LAND MANAGEMENT HANDBOOK

40

Field Studies of Seed Biology

1997

BRITISH COLUMBIA
Ministry of Forests
Research Program

Field Studies of Seed Biology

Carole L. Leadem, Sharon L. Gillies, H. Karen Yearsley,
Vera Sit, David L. Spittlehouse, and Philip J. Burton

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INTRODUCTION

I like trees because they seem more resigned to the way they have to live than other things do.
(Willa Cather "O Pioneers!")

Except in limited areas where there is enough advance regeneration, establishment of forest cover on harvested lands continues to depend on seedling planting programs or on natural regeneration by seeds. Whereas successful plantation programs depend primarily on plant competition and site variables at the time of planting, successful natural regeneration depends not only on the availability of seeds, but on favourable environmental conditions throughout the processes of seed production, dispersal, germination, and seedling establishment.

Site preparation and other silvicultural treatments can improve the suitability of the seedbed and its micro-environment, but there is still much we do not understand about how various factors contribute to successful forest establishment. We have gained some insights, under controlled conditions, about the influence of major factors such as light and temperature, but we have limited experience with biological responses under actual conditions in the field.

Anyone who has conducted research in the field quickly comes to realize the complexity of the systems chosen for study. An immense number of external and internal factors that affect living organisms must be taken into account—with limited possibilities to control these factors. A major constraint, particularly in a forest environment, is the difficulty inherent in conducting field studies involving seeds. Infrequent seed production, predation by animals, difficulty locating small seeds, estimating the numbers of buried seeds, measuring germination, and monitoring survival pose myriad challenges for the field researcher. Added to these difficulties is the lack of information about effective methods for conducting field studies of tree seeds. A recent assessment of ecosystem management needs stressed the importance of standardized sampling and monitoring techniques, and the lack of consistent methods for archiving, accessing, and updating databases (U.S. Dep. Agric. For. Serv. 1996a). Techniques gleaned from agriculture literature are generally not applicable, and traditional ecological studies (e.g., of seed banks) tend to be primarily descriptive with little emphasis on experimental approaches.

The primary objective of this manual is to detail methods that have been gleaned from the literature and from personal experience of the authors. It is a manual of methods with some general guidelines and interpretation. Relevant background papers are cited where appropriate, but it is not a literature review. The manual is intended for use by researchers in public and private forest resource management agencies, universities, and colleges. Although specifically directed to tree seed research in forested ecosystems, many of the methods described can be used to study seeds of graminoid, herb, and shrub species in both forest and non-forest plant communities. The extensive background information included in the text also provides valuable reference material for many who have an interest in tree seeds, but who are not directly involved in research activities. The detailed examples from previous studies are included, not to prescribe how such studies should be done, but to assist in planning by providing reference values on which to base measurements, sample sizes, and other experimental details.

Since the manual is directed primarily to researchers working in the province of British

Columbia (B.C.), Canada, many examples (forest types, species, research topics), procedures (the biogeoclimatic ecosystem classification system), and regulatory policies are specific to this geographic and political jurisdiction. Nevertheless, it is hoped that the underlying principles are self-evident and will be generally applicable to the conduct of field research elsewhere.

ORGANIZATION OF THE HANDBOOK

Following a discussion of planning and organizing a field study (Section 1) and setting up an environmental monitoring program for the experimental site (Section 2), the manual is arranged by subject areas most often associated with field studies of tree seeds: natural seed production (Section 3), seed dispersal (Section 4), seed predation (Section 5), seed banks (Section 6), assessing seed quality and viability (Section 7), and effects of silvicultural practices on emergence (Section 8). Each section was written by one or more experts as follows:

Carole Leadem, Ph.D., R.P.Bio., earned her degree in plant physiology from the Botany Department, University of British Columbia, and is a member of the Association of Professional Biologists of British Columbia. She has been in charge of the tree seed biology research program with the B.C. Ministry of Forests in Victoria since 1978. Carole Leadem wrote the sections on planning and organizing field studies (Section 1), natural seed production (Section 3), seed responses to the environment (Section 7.1), seed testing in the laboratory (Section 7.2), seedbed preferences (Section 8.3.1), and contributed to the sections on seed dispersal and silvicultural practices.

Sharon Gillies, Ph.D., earned her degree in plant physiology from the Department of Biological Sciences, Simon Fraser University. She has been a biology instructor at the University College Fraser Valley since 1995. Sharon Gillies coordinated compilation of the original manuscript, was responsible for creating the handbook structure and adhering to Ministry of Forests style manual, edited author submissions for the first complete draft, wrote the section on seed dispersal (Section 4), and provided environmental monitoring material for Section 2, and Table 8.1 on seedbed suitability.

H. Karen Yearsley, M.Sc., R.P.Bio., earned her graduate degree from the Faculty of Forestry, University of British Columbia, and is a member of the Association of Professional Biologists of British Columbia. Her 15 years of research experience in B.C. include work on ecosystem classification, forest succession, and forest soil seed banks. Karen Yearsley wrote the sections on seed predation (Section 5) and soil seed banks (Section 6), and contributed to the sections on planning field studies (Table 1.1) and seed dispersal (Section 4).

Vera Sit, M.Sc., earned her graduate degree from the Statistics Department, Dalhousie University, and is a member of the Statistical Society of Canada. She has been with Biometrics Section, Research Branch, B.C. Ministry of Forests, since 1990. Vera Sit wrote the sections on experimental design and data analysis (Sections 3.6, 3.7, 5.5, 6.4, 6.5, 7.4, 7.5) and the case studies (Section 3.8), and reviewed and contributed to all the statistical sections.

David Spittlehouse, Ph.D., P.Ag., earned his graduate degree in forest climatology from the Department of Soil Science, University of British Columbia. His research includes modifying site microclimate to improve seedling regeneration, and determining how forest harvesting and regrowth of the forest affects forest hydrology. He has worked for the B.C. Ministry of Forests in Victoria since 1982. Dave Spittlehouse wrote most of the section on designing an environmental monitoring program (Section 2).

Philip Burton, Ph.D., R.P.Bio., earned his degree in plant biology from the University of Illinois at Urbana-Champaign. An independent researcher and consultant, he has been investigating seed biology, forest regeneration, and vegetation dynamics since 1979. Phil Burton contributed material for the

sections on seed dispersal (Section 4), field germination studies (Section 7.3), and effects of silvicultural practices (Section 8).

Each section contains background material on the subject and descriptions of some of the methods and approaches that have been used. There is also advice on experimental design and analysis of the data. Some laboratory procedures have been included to serve as controls for experiments conducted in the field. Laboratory experiments can provide valuable data to supplement field measurements because the results are generally reproducible and environmental variables can be controlled. Many terms are discussed in a comprehensive glossary, and the main subject areas have been indexed.

The logistics of field research are difficult enough in their own right. We hope the information contained in this handbook will help those contemplating new research projects to avoid some of the pitfalls associated with studies in the field. We anticipate other benefits: that this handbook will help standardize field methods and enable comparisons between studies, will increase cooperation between investigators, and will promote more efficient use of resources (equipment, finances, personnel). All of these efforts will help broaden the forest resource database and increase our understanding of the multiplicity of factors involved in forest regeneration.

We anticipate that methods documented in this handbook will be improved once they undergo more extensive field testing, and we invite comments about the information and methods suggested here, and about your own field experiences. Please direct your suggestions to the senior author at the address inside the front cover.

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CONTENTS

APPENDICES

FIGURES

TABLES

SECTION 1 PLANNING TREE SEED RESEARCH IN THE FIELD

The road to chaos is paved with good assumptions.
(Anon.)

It is much less expensive to learn from other people's mistakes than your own.
(McRae and Ryan 1996)

1.1 Overview

Forests cover only about 11% of the earth's surface, yet they account for nearly half of its net primary productivity, and about 70% of the net productivity occurring on land (Whittaker and Likens 1973). Forest ecosystems are complex and diverse, and develop relatively slowly. Thus, forest ecosystem research projects may require years to produce meaningful findings that can be widely applied.

The duration of a study depends entirely on what you want to find out. Many of the biological, physical, and chemical phenomena associated with forest ecosystems can be studied over relatively brief periods on temporary field plots or in the laboratory. To document and compare natural processes, short-term descriptive and baseline studies are essential. Short-term studies also are important to establish the immediate impact of processes on a system, even though they are prone to being confounded by environmental fluctuations such as climate.

On the other hand, many biological phenomena, such as plant succession, occur on time scales of decades or centuries. Long-term studies allow us to evaluate interactions among the various factors controlling ecosystem function that, on a short time scale, might seem inconsequential. Forest ecosystems usually require many years for the effects of perturbations to subside and for long-term trends to appear. This is especially true for communities not in equilibrium, such as those recovering from fire or harvesting.

1.2 General Structure of Successful Field Studies

A survey of long-term forest research programs conducted at many locations throughout the world by Powers and Van Cleve (1991) stressed the importance of planning, commitment, and focus. They concluded that successful long-term experiments shared eight essential components. Not all field studies are conducted over long periods, nonetheless, consideration of the following principles is instructive to anyone contemplating field research, regardless of duration.

1. *Sustained commitment*
Fluctuations in philosophy, politics, and funding are the surest way to dampen scientific spirit and inspiration. Field studies, once established, must have a fair certainty of continued support, at least to the level required to maintain research sites and to collect core data. This support should be free from political interference. To enlist this level of commitment, researchers should present their arguments based primarily on the benefits that can be derived from investigating socially relevant issues (e.g., sustaining wood production, providing clean water, protecting soils). Proposals that are couched in terms of "understanding how forest ecosystems work" are far less likely to be granted support by funding administrators.

2. *Long-term dedication of a site*
Plots maintained after the original questions have been answered can continue to have demonstration

value for professionals and the public, and can pay substantial dividends well beyond the life of the original study. Again, the chances of having land dedicated for the site will be enhanced if the research has a central, timeless theme. Support is more likely to continue if research results are disseminated rapidly to administrators and land managers.

3. *A guiding paradigm*
A central focus is necessary to provide structure and maintain research objectives. As long-term objectives may grow hazy with time and personnel changes, periodic reference to the guiding paradigm will help to refocus the research.

4. *A central hypothesis*
A clear statement of the principal scientific question that the research is designed to answer helps to clarify the research direction and stimulate development of the experimental approaches. The central hypothesis is tested through a number of individual studies with definite life spans that terminate once a particular question has been addressed.

5. *Large plots and replication*
Plots should be large enough to simulate natural ecosystem conditions as closely as possible. Large plots not only minimize edge effects, but also increase the flexibility of future studies on research sites. Options might include retaining extra control plots that could later be converted to secondary treatments, or creating split-plots for treatments supplemental to the original design. Large plots facilitate replication of treatments, which is essential for statistical analysis and setting confidence intervals.

6. *Interdisciplinary approach to research*
Field installations should be made available to all research collaborators, regardless of affiliation or specialty. This will attract excellent scientists and promote openness and synergy. Studies that attract a broad array of scientific interests result in much greater understanding than can be achieved through isolated, independent efforts. In addition, program scientists benefit from exchanging ideas, cooperating on experimental work, and collaborating on professional papers. Because collaborators have an interest in continuity and maintaining the research site, interdisciplinary field studies inherently promote the stability of long-term projects. However, interdisciplinary studies only work if there is strong central planning and coordination.

7. *Extension of results*
Research must be designed to make data as portable as possible so that results can be generalized to a variety of species, soils, and forest types. Research will have the highest value if results can be incorporated into a network of coordinated, but geographically separated, studies. Experiments should be sufficiently comparable so that databases can be shared, and each research site should be instrumented so that a baseline of climatological data can be established.

8. *Low red tape*
Maintain the least amount of bureaucratic structure needed to prevent chaos. Initially, a board of senior scientists from a variety of disciplines should review all research proposals. Later, a research coordinator can review projects to ensure that one study does not interfere with another, and that all collaborators are kept abreast of the overall research program. Depending on the size of the research site, a site manager may be needed to facilitate day-to-day (or seasonal) scheduling.

1.3 Designing a Field Study

Careful initial planning and organization are critical to the success of any field study. Considering the complexity, the expense, and the duration of many field studies, the consequences of poor planning can be great.

Most studies consist of three stages—planning (Stage I), data gathering (Stage II), and data analysis and interpretation (Stage III). However, the framework for all three stages is constructed during the planning stage. The planning process can be articulated as a series of steps that provide answers to the questions: *why*, *what*, *how*, *when*, *where*, *how much*, and *so what*.

1.3.1 Formulating the hypothesis

The first step before undertaking any field study is to formulate a clear statement of the principal scientific

question or central hypothesis that the research is designed to answer—the *why* of the experiment. A research plan with a clear statement of the problem helps to identify the research direction and provide valuable guidance if the project should run into difficulty (such as loss of support, changes in personnel, or environmental disaster).

1.3.2 Stating the objectives

Once the principal scientific problem has been elucidated, research objectives provide the necessary structure for planning and executing the project. Objectives are succinct summary statements of *what* the research is trying to achieve. Keeping research objectives in focus during all stages of planning will stimulate development of experimental approaches, guide complete and efficient collection of data, and keep the research on track.

1.3.3 Selecting the factors to study

The factors to be studied—another aspect of *what*—are usually specifically identified in the statement of objectives. The factors chosen will depend upon whether the study is primarily descriptive or experimental in nature. In experimental studies, factors are the vehicles through which the objectives are achieved, and they are generally identified as treatments. Factors may consist of one or several levels. For example, suppose your objective is to determine if light affects the survival of lodgepole pine germinants on open harvested sites. To investigate this objective experimentally, you would identify *light* as the factor to be tested. You might also want to more specifically compare how different light levels affect seedling survival; then you would expand the light treatment to include several levels, such as full sun, partial shade, and full shade.

Constructing a schematic diagram of the biological cycle (or other process) is an effective way to identify what variables affect the process being investigated. Diagrams help to clarify relationships and suggest the most appropriate factors to study (Figure 1.1). For example, if you want to study initiation of reproductive buds, you will want to include climate, plant condition, and resource availability as major factors in the experiment. On the other hand, if you want to examine the factors affecting field germination, the most suitable variables to study would be (micro)climate, substrate, and species.

Treatments should be chosen to reflect major changes in ecosystem function. Viewing ecosystems under extreme conditions is most likely to reveal how various ecosystem components function and to demonstrate the capacity of these components to recover from change. Studies will have greater value if the treatments have a generally continuous pattern (i.e., increasing or decreasing in size). Data obtained from such treatments lend themselves to predictive regression analysis. Changes in responses can then be correlated with changes in the magnitude of specific factors, and results can be more readily extrapolated to similar sites. Choosing treatments that span and extend slightly beyond the full range of expected responses helps to define the end points and establish the limits of the system under study.

Particularly in long-term studies, it is advisable to retain some flexibility in the original design by incorporating ways the experiment can be changed if future circumstances should require it (Leigh et al. 1994). To ensure the longevity of the field site, it is best if only minimum changes are made to the treatments. Changing the experiment to obtain more information in the short term generally results in sacrificing the longevity of the treatments. It is sometimes advantageous to incorporate innovations in forest management practices into the study, but this should be done only if the major objectives can be retained. Another possibility is to modify the original objectives and continue the experiment in a different form, but again longevity will be lost. A final, but generally less desirable option, is to set aside the site indefinitely or reserve it for future use.

1.3.4 Selecting the methods

Methods can be considered the *how* of experimental studies. The techniques chosen for the study will be governed by the study objectives, and the most effective means of achieving those goals. Usually, more than one method will achieve a particular purpose, and for this reason a variety of methods for field studies of tree seeds has been included in this handbook. Ultimately, the choice of the most suitable technique will depend on the available resources. In most cases, the final decision will be based on

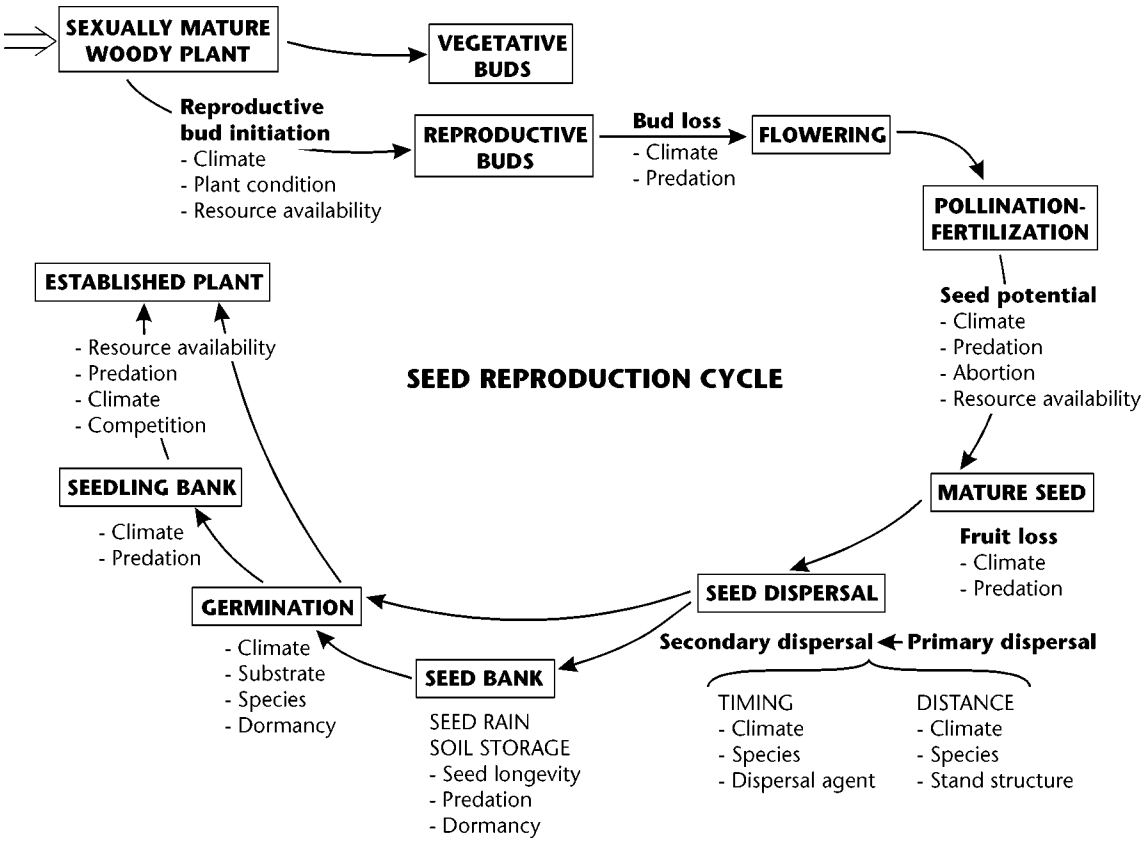


FIGURE 1.1 *Framework for evaluating the seed reproduction process in boreal forest trees (adapted from Zasada et al. 1992). A schematic diagram can help to clarify processes and suggest factors for the study.*

balancing the trade-offs between the detail desired and the constraints of time and money.

Most research projects will employ a variety of methods. Often the distinction between different methods is determined more by the purpose than by the type and intensity of the monitoring. Note that different objectives do not necessarily require distinct and independent data collection efforts. There may be some overlap in the data needs. As long as the research objectives are kept clearly in mind, taking advantage of this overlap can result in substantial cost savings.

Answers to the following questions will help in choosing appropriate methods: What is the primary purpose of the measurement? How well does the chosen method quantify the factor or characterize the intensity of the response? How precise or accurate is the method? How many measurements are required? If many or frequent measurements are required, can the process be automated? The choice may also be affected by the logistics of the experimental site—certain techniques may not be suitable for field use. For example, precise and automated methods may be ideal for making a particular measurement, but the instruments may not be robust enough for field conditions or may require an external source of reliable power.

After choosing the methods, it should be determined whether the research data are continuous or categorical. The term *continuous* implies that the measurements, in theory, belong to a numerical scale consisting of an infinite number of possible values. However, sometimes you need to measure a quality or condition that cannot be expressed on a continuous scale. This discrete, noncontinuous type of data is called categorical because it generally consists of the number of observations falling into prespecified classifications, groups, or categories (e.g., number of seeds that have or have not germinated).

Continuous data can usually be analyzed using parametric methods such as ANOVA, regression, and MANOVA (Sections 6.5, 7.2.5). However, nonparametric analysis is sometimes required for continuous data if the assumptions of ANOVA, for example, cannot be met. Categorical data, on the other hand, are usually analyzed using methods such as contingency tables and log linear models (Sections 5.5, 7.2.5) although alternative nonparametric techniques are available, if necessary.

Data can also be categorized by the type of measurement used for collection. Determining the type of measurement needed helps to identify the type of information required, the frequency of data collection, and the most suitable means of analysis. Types of measurement include assessment, inventory, monitoring, and visual data.

An *assessment* is an estimation or evaluation of the significance, importance, or value of a quality or character. It generally implies a subjective judgement (e.g., maturity) to determine placement in a class. The classification scheme may be based on some arbitrary characteristic or a ranked order. Assessment data are usually nonparametric.

An *inventory* is an itemized list or catalog that may or may not be organized into groups. Usually the number of items in a group are simply counted, with no additional judgement or interpretation. For example, for an inventory of seeds in a seed bank, a count is made of the number of seeds by species present in the soil. An inventory is usually a one-time measurement, but it can be repeated periodically (e.g., annually). Greater use often can be made of inventory data if the samples are stratified in some way, for example, by making separate seed counts at various depths of a soil core, rather than performing a single count of the total core. Depending on the manner in which samples are taken, inventory data can be parametric or nonparametric.

The term *monitoring* is used to describe a series of observations made over time. The repetition of measurements to detect change over time is the quality that distinguishes monitoring from the related processes of inventory and assessment. The data obtained can be parametric or nonparametric. MacDonald et al. (1991), in compiling guidelines for monitoring water quality, recognized seven types of monitoring: baseline monitoring, trend monitoring, implementation monitoring, effectiveness monitoring, project monitoring, validation monitoring, and compliance monitoring.

Visual data are another significant source of primary scientific information, although they are not generally considered as data. Visual representations may be the most effective way to present information that otherwise would be too unwieldy or difficult to understand (e.g., site maps or structural diagrams). Some information can only be captured visually (e.g., seed X-rays or photomicrographs of plant structure). Although not quantitative, visual data represent an important source of research information, and a valuable means of portraying certain characteristics. Unfortunately, visual data are underutilized in most research studies.

1.3.5 Setting the time frame and determining a schedule

Before starting the study, a schedule should be prepared to outline the temporal distribution of the major components of the study. This is the *when* of experimental studies. Many field studies are short in duration, but some studies can be very lengthy, such as the ecological studies of the Carnation Creek watershed on Vancouver Island, which have been under way for over 20 years.

The experimental design and type of data analysis will direct how often to collect the data (e.g., daily, weekly, monthly), but the timing of treatments must also be taken into consideration when designing field studies. Treatments may be applied only once, repeated at fixed periods (e.g., annually), or even rotated. The timing will also depend on what type of information is required—whether you are interested in the direct effects in the year the treatment is applied, the residual (or carry-over) effects in subsequent years, or the cumulative effects of repeated treatments.

The length of the study periods should be clearly defined, especially when planning a long-term study. The most suitable period length is defined by how long plot management can be kept constant. Period length might also be governed by the time when the first full assessment can be made (e.g., at the end of the first growing season), or when treatment differences might first be discernible.

In some instances, period length may be used to apportion temporal variation (McRae and Ryan 1996).

In the same way that blocks are used to control spatial variation among plots within a site, changes over time can be partitioned into periods. Although period lengths may sometimes differ because of operational constraints, analysis is simpler when all plots have study periods of the same length.

1.3.6 Choosing the test conditions

The next step is to determine *where* the factors will be tested. Depending on the research objectives, the test conditions are sometimes considered to be a factor of the experiment. If this is the case, you should choose test sites or conditions that follow some sort of progression or gradient (e.g., small to large openings, low to high elevation). This will allow the results to be more readily generalized to other sites (if the requisite experimental design criteria have been met).

Most details relating to test conditions will be specific to the study and what you are trying to ascertain. For further details refer to the section of interest: seed production (Section 3), dispersal (Section 4), predation (Section 5), germination of seed banks (Section 6), laboratory and field germination tests (Section 7), and silvicultural practices (Section 8).

1.4 Experimental Design

If you don't deal with each of these levels of variation, your sampled population may not be representative of your target population, and in that case a statistician or a sharp lawyer can make you and your data look pretty lame.
(MacDonald and Stednick 1994)

Once the objectives are identified and the factors, methods, and test conditions are established, attention should be turned to experimental design. The experimental design will prescribe how essential elements of data collection (Stage II) and data analysis and interpretation (Stage III) are executed. Field studies require substantial commitments of time, labour, money, materials, and maintenance; inadequate attention to details such as experimental design and data management can pose considerable risks to the resources invested in the project. Losses due to errors in experimental design may severely damage a scientist's reputation and will reflect badly on collaborators.

1.4.1 Basic concepts

It is assumed that readers of this handbook have some knowledge of statistics, and will know where to obtain assistance for particular statistical problems. The statistical discussions included in various chapters are intended only to provide general background on important aspects of experimental design and data analysis, and to raise awareness of some potential pitfalls or problems that may be encountered in specific topic areas. Discussions relating to some common statistical methods can be found in the following sections: summary statistics (Sections 4.5.1, 6.5); ANOVA (Sections 3.7, 4.5.2, 6.5, 7.5); regression (Sections 4.5.3, 6.5, 7.4); correlation (Section 3.7); and chi-square (Sections 5.5, 8.3.4).

Careful study of the proposed designs can be invaluable during the planning stage (McRae and Ryan 1996). Trial analyses will demonstrate whether the contrasts of interest can be estimated, and will point out deficiencies in the design and analysis methods. A postmortem of similar experiments often provides data sets and estimates of experimental errors that can be used to evaluate the proposed design. As in the actual experiment, there should be sufficient replication to achieve the degree of precision required to detect the treatment differences. If trial analyses reveal it is unlikely that differences will be found, the study may not warrant the investment. This is especially critical for long-term studies.

The distribution of replicates in space and time is the most critical element of experimental design. Randomization provides for estimates of the experimental errors, which should always be reported, either as the standard error of the mean or the difference between means (McRae and Ryan 1996). Replication is generally accomplished by applying treatments to two or more plots within the site (often divided into blocks) and/or by repetitions of the experiments at other locations or times.

The allocation of replicates will be largely a function of the objectives and the expected variability (MacDonald and Stednick 1994). The more sources of variability you address, the more reliable your results will be. In general, it is not efficient to test for all sources of variability everywhere. However, if you do not repeat any of your measurements, you have an unknown source of error that will weaken all your subsequent conclusions. Repeated measurements

can give a better estimate of a variable such as seed production or field germination, but when you are replicating your measurements, you have to be clear about the level of variability with which you are dealing. Measurement variability is very different from the variability between experimental units (e.g., field germination plots).

Each time you design a project, you need to identify all these potential sources of variation, and determine how you want to deal with them. If you don't deal with each source of variation, your sampled population may not be representative of your target population. Hurlburt (1984) uses the term pseudoreplication to refer to the testing of treatment effects with an inappropriate error term for the hypotheses being tested. You can think of it as a source of variability which is inherent in the data but cannot be defined because of the sampling strategy. In other words, if you have some sources of error in the data that cannot be tested, then typically you are dealing with pseudoreplication.

One of the examples Hurlburt (1984) uses is a study to determine the effect of water depth on the rate at which leaves rot in a lake. Although this is not a seed-related example, it is worthwhile using here because it is so clear and succinct. Four bags of leaves were placed together at one location at a depth of 1 m, and another four bags were placed together at another location at a depth of 10 m. After some time the bags were retrieved, dried, and weighed. If there was a significant difference between the two sets of bags, all that can be said is that there was some difference between the two locations. To make an inference about the effect of a particular water depth on leaf rotting in this lake, the bags would have to be distributed at the same depth around the lake. To make a more general statement, replicated samples would have to be distributed at different depths in several lakes. The design depends on the question you want to answer, but placing all the bags in one place is pseudoreplication. From Hurlburt's point of view, you must have replication on at least one level. If you don't have the ability to test for differences, it is not an experiment.

Often you need to make statistical compromises, and if so, you should be explicit about the statistical trade-offs that you have made, rather than letting them be set by neglect or default (MacDonald and Stednick 1994). For example, in natural resource management, the significance level is typically set at $\alpha = .05$, meaning that there is a 1 in 20 chance that an observed difference will be due to chance. A strong level of significance combined with high variability means that usually you will not detect a statistically significant change until damage to the resource has occurred. However, given the high natural variability in natural systems, it may be better to use a less stringent significance level in exchange for a higher level of resource protection. Another example is power, which is usually designated as $1-\beta$. When comparing two sample means, the quantity β is known as a Type II error, which is the probability of incorrectly concluding that two populations are the same when in fact they are different. Again, if a resource is slow to recover or is of high value, you probably want to increase the value of α. In natural resources management α should probably be set at .10 (MacDonald and Stednick 1994).

Excellent discussions of pseudoreplication can be found in Stewart-Oaten et al. (1986), Bergerud (1988), and MacDonald and Stednick (1994). Additional discussion of randomization and replication in relation to field studies of tree seeds can be found in Section 7.4.3.

Blocking of the plots reduces experimental error by removing any gradient effects in site variation. Choosing blocks that are arranged contrary to the field gradient will increase the experimental error, but this is difficult to avoid because the field gradient is often unknown. Appropriate variables on the site (temperature, soil, moisture) can be measured and the results used as a basis for blocking. If elevations of the site vary considerably, blocking would most likely be parallel to contour lines, and not perpendicular. The effectiveness of a block arrangement can be assessed only after the experiment has been run. A good strategy is to have a robust blocking structure that allows for an environmental gradient in either or both directions in a rectangular site layout (McRae and Ryan 1996). See also Section 7.4.

In forestry research the same unit or process is usually measured on more than one occasion. For example, in trials to compare several treatments, data are typically collected before and after treatments are applied. Such data tend to be serially correlated, or autocorrelated, which means that the

most recent measurements are dependent on, or to some extent predictable from, earlier observations. Because this violates the independence assumption on which many standard statistical methods are based, alternative methods are required for their analysis. Two broad classes of methods have been developed for this purpose: repeated-measures analysis and time-series analysis. For additional background and discussion of this topic, refer to Nemec (1996).

Carry-over effects from previous treatments are a hazard of long-term studies in which multiple treatments are applied. When analyzing by ANOVA or multiple linear regression, estimates of the direct effects of later treatments must be adjusted for any residual effects remaining from previous treatments (McRae and Ryan 1996).

1.4.2 Determining the sample size

To ensure that enough samples are collected for a study—the *how much* of the experiment—it is advisable to determine the appropriate sample size specific to the parameter being studied.

Sample sizes for each measurement must be determined independently, because variability may be different for different characteristics. For example, the sample size required for measuring cone characteristics may not be the same as that needed for seed characteristics, even for the same species, because of differences in the variability of the data (Carlson and Theroux 1993). Environmental changes may also result in year-to-year variations, but these differences can sometimes be minimized by adjusting all values to be relative to those observed in a particular year (Ager and Stettler 1983).

Sample size is usually determined by applying statistical efficiency calculations to a preliminary set of measurements (Sokal and Rohlf 1981; Ager and Stettler 1983). See also Stauffer (1981, 1982) for sample-size tables oriented to forestry applications. In the absence of any other information, a sample size of 10 is often a good place to start (MacDonald et al. 1991; MacDonald and Stednick 1994).

The topic of sampling, both how to sample and how many samples to collect, is a critical aspect of all field studies of tree seeds. For more detailed discussions relating to particular subject areas, see Section 3 for seed production, Section 4 for seed dispersal, Section 6 for seed banks, and Section 7 for seed germination tests. A more general discussion of various types of sampling can be found in Cochran (1977) or Thompson (1992).

1.5 Data Management

Data management protocols should be established when the study is initiated. The type of data, experimental design, and method of analysis will guide how the records and data are organized and managed. This section provides a brief overview of the major points of data management, as well as some special considerations required for long-term studies.

1.5.1 Establishing a coding scheme

A consistent coding scheme should be established to correlate all data records with the research plots and treatments. The coding scheme is best defined in a table assigning unique label codes to identify field plots, factor levels, treatments, and replications. The table should indicate the exact units in which the data will be recorded (e.g., millimetres, kilograms, or watts per square metre). For categorical data (Section 1.3.4), a brief description should be given of the significance of each classification code (e.g., in Section 3.5.1, for cones, 1 = scales fully open; 2 = scales partly open; 3 = scales completely closed).

Allow for some flexibility in the coding scheme so that labels can be added if new treatments are incorporated, or if treatments change over time. If treatments are changed, ensure that the coding scheme is annotated to relate the new treatments to the original treatments.

The same format should be established for field and computer records so that data can be easily accessed for future examination or analysis. Where feasible, coordinate with other agencies or researchers to use standard codes or data-entry protocols. This will facilitate exchange of data between programs. For example, the standard coding formats used for the biogeoclimatic ecosystem classification data should be used for all site and vegetation data. Standard species names and codes for British Columbia can be found in both ACCESS 2.0 and EXCEL 4.0 files at the B.C. Ministry of Forests Research Branch FTP site (see Appendix C) in the directory/pub/provspp. The files are regularly revised and updated. If you want

to collect vegetation data, then you should follow *Describing Terrestrial and Aquatic Ecosystems in the Field* (in preparation 1997) which will update Luttmerding et al. (1990). This is also a useful reference for making site descriptions (see Section 1.6.5). A variety of computer data entry and reporting tools (e.g., VENUS) are also available (see Appendix C for more complete information).

1.5.2 Creating a permanent file

The permanent file should include the initial plans and objectives and all parameters of the experiment. A statistical guide should be included in the permanent file giving full details of the experimental design(s), the proposed method of analysis, and all associated computer programs. Include the type and number of annual data sets, and a list of the different annual records. Special notes about the trial should be recorded and arranged by date or other logical sequence. Include maps giving details of the research sites, the location of the plots, and the arrangement of the treatment blocks and replications (see Figure 1.2).

Provide room in the structure of the permanent file so that you can add data and field notes for the current year and update the parameters, if necessary. It is useful to link computer data files to a spreadsheet or graphics program to produce a series of graphs depicting the different responses over time for each treatment. Create a summary table of the cumulative effects for each variate, giving the relevant summary statistics.

Plan the permanent file so that computer formats and files will remain compatible over changes in computers and software. To ensure efficient data entry, carefully design data sheets and format computer files. The spreadsheet software into which you plan to import your data should guide the data file format. For most data, a row/column format is best. If you are uncertain about the type of software that will be used, a simple ASCII (text) file format is recommended.

The permanent file should also contain detailed directions for finding the plots again after installation. The importance of this step cannot be emphasized enough, especially if different people are resampling the plots. Several scales of maps are needed to relocate plots. Section 1.6.4 includes a more detailed discussion of recording site parameters for relocation.

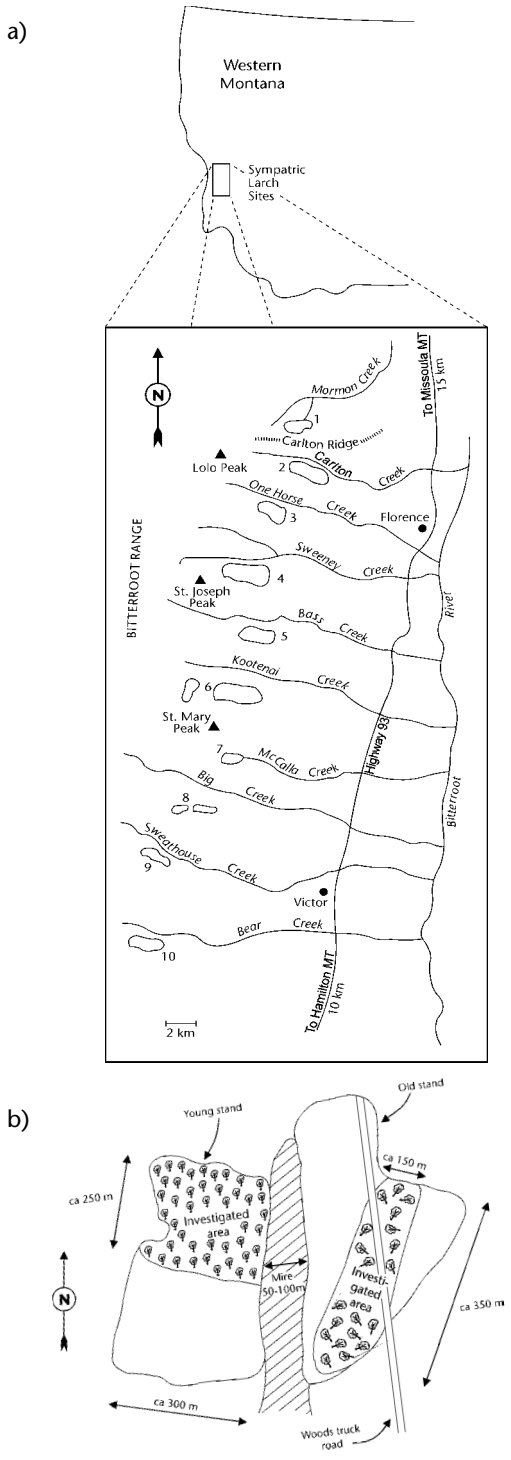


FIGURE 1.2 *Maps giving details of the research site should be included in the permanent file. (a) Locations where western larch and subalpine larch are sympatric (Carlson and Theroux 1993). (b) Sketch of investigated stands of* Pinus sylvestris *(Bergsten 1985).*

1.5.3 Preparing to collect data and samples

Computer-generated administrative aids (labels, data sheets, random order lists) will simplify data and sample collection. Organic tissue and soil samples collected during the study must be properly coded and archived for analysis or future reference. Pre-printed labels simplify collection of samples in the field and act as an additional check that coding sequences are complete. Colours and symbols (e.g., stars, circles, triangles) used in addition to, or instead of, numerical codes will help to reduce errors, which may result from performing repetitious tasks under arduous field conditions.

The sequence prescribed by the randomization scheme can be used to arrange labels, sample containers, and data sheets. If you have a large number of items, it may be convenient to subdivide them into smaller groups (e.g., by plot number).

Permanent markers (stamped metal or plastic are best) should be generated for all treatments, and securely attached to durable, highly visible posts or other stationary devices in the field plots. For further details, see Section 1.6.4, Table 1.1.

1.5.4 Collecting the samples and recording the data

Data can be recorded in the field using manual records or hand-held dataloggers or other automatic recording apparatus. Pre-labelled sheets can be used for manual data entry, or datalogger files can be pre-programmed with plot, treatment, and sample codes. Refer to Spittlehouse (1989) and Section 2.2 for additional information on using dataloggers in the field.

Transfer of data is now relatively easy using computers and computer interface devices, but all files must be regularly backed up to avoid loss of data. You should have spare power sources, in case primary equipment fails.

1.5.5 Reporting

A complete analysis of the research and a summary report should be prepared annually or at the end of each field season. This can be considered the *so what* of the experiment—what do the results mean in the greater scheme of things. Strive to disseminate as quickly as possible the interim results or updates at technical meetings, in short articles, or in newsletters. Prompt reporting will help maintain support while the research is in progress.

1.6 Selecting and Describing the Study Site

1.6.1 Selecting the study site

An essential part of planning is selecting a suitable site—the *where* of the experiment. Site selection should take into consideration practical aspects such as accessibility, the frequency of site visits, and how seasonal changes may affect access and any permanently installed instrumentation.

As early as possible in the planning process, contact the local forest district or the forest management office responsible for the proposed study area. Establishing a good relationship with those ultimately responsible for the site can have unexpected benefits and will also serve to promote your research amongst the forestry community. Local staff may be able to suggest potential sites that meet your criteria and provide more detailed information if they know the objectives and the key factors you wish to study. From them you can gain considerable information and knowledge about local forestry practices that will directly and indirectly influence your work or research, now and in the future. Because they are located near the site, they may be able to maintain security or assist with site maintenance. In addition, if local forestry staff know about your study, the chances of the trial being damaged by concurrent industrial or silvicultural activities is greatly reduced.

The scientific criteria for selecting a site depend on the goal of the study, but all critical site-related factors must be identified. For example, if the objective of the study is to determine the difference in seed production between north- and south-facing slopes, then site selection will be dictated by the aspect and grade of the slope. In other studies, slope and aspect would not be primary factors. If the goal is to describe seed production in mixed stands as opposed to uniform stands, then the primary selection criteria would be the species composition of the stands. For long-term research on seed production in a natural stand, it would be important to locate each site away from openings or roads. In this case, a fixed area on each site might be delineated in the centre of the stand, with the trees surrounding the plot acting as a buffer zone to reduce edge effects.

Site illustrations are useful in documenting the key elements of the field site (Figure 1.2), and should form part of the permanent file (Section 1.5.2). They

may also be used to find the plots again for repeated measurements (Section 1.6.4).

1.6.2 Deciding on temporary or permanent plots

A decision must be made whether to use temporary sites (entirely new units are randomly selected for observation each time), or permanent sites (the same units are observed repeatedly over time). The choice of temporary or permanent plots depends on the degree of correlation you expect between the initial and final plot values. If a high positive correlation is desired, permanent plots will generally give better precision. If large-scale changes are expected in the nature of the site, temporary plots should be used (Freese 1962). Sometimes a combination of temporary and permanent sites can be used—permanent (intensive) sites for detailed aspects of the study and temporary (extensive) sites for broadening the number of samples and site types.

1.6.3 Determining size and shape of the plots

The size and shape of the plot depend on a number of factors, including the goal of the research, the cost or time required for sampling, the required precision, and the uniformity or heterogeneity of the area (Freese 1962). There are obvious trade-offs between plot size and homogeneity of samples. You want as large a sample size as possible, with good treated buffers, but the larger the plot size, the more likely you are to introduce heterogeneity (in soils, nutrient regime, moisture regime, slope, etc.) into your plot selection, thereby increasing the within-plot error sources. To assess homogeneity, a full site description of each plot is recommended. Plots should only be accepted for inclusion in the study if the variation in site type would not compromise the long-term results. In general, moving more than one full site series (or other environmental gradient) within a plot is probably sufficient reason to abandon it. Larger plots (more than 40×40 m) should have multiple soil pits to ensure homogeneity.

The duration of the study will also govern plot size. If there is any possibility of continuing the study for 5 years or more, consider the plot size carefully. For example, if your original objective was to study germination and initial development, but later you decide to extend the length of the study, you may be unable to do so if the plot size initially chosen was too small.

The choice of plot size often depends on stand density and heterogeneity. To compensate for differing stand densities or species mixes in a study, the plot size could vary to maintain a constant number of trees or species types within each plot. See Smith et al. (1988) for an example of this approach.

Another approach for studying tree density effects is to use rectangles of fixed dimensions (width and length) for all sample areas. Tree density can be estimated by dividing the number of healthy trees within a sample rectangle by the area of the rectangle. This approach is preferable because the fixed dimensions provide consistent estimates of site variation across all study areas, and ensure the validity of tests for tree density effects.

1.6.4 Installing, marking, and relocating the plots

Once the experimental site has been selected, the plots must be identified and the boundaries clearly marked. Markings must be highly visible and durable. The choice of marking method will depend on a number of site factors such as the distance from the road, steep terrain, annual snowpack, rocky ground, or height of vegetation. The durability required of markers will depend on the amount of exposure to the elements, the possibility of crushing or toppling by large animals (bears, moose, cows, humans), and the total length of time the plot will be sampled. A summary of important points for installing and marking plots is given in Table 1.1.

After installation, the site location should be recorded in detail in the permanent file. This is an important step, and will prove particularly valuable if the plots are resampled by different people or over many years.

- *To locate the general vicinity of the site*: Mark site locations on topographic maps, forest cover maps, airphotos, orthophotos, etc. Write out directions including distances (km) to each turning point and road names, etc., from likely starting points (towns). Use GPS locations if you can afford and have access to this technology.

- *To locate the site from the point of access (e.g., road)*: Draw a site map of the plot(s) and surrounding area with local landmarks (e.g., roads, water, rocks, slopes, directions, etc.). If there is more than one plot, ensure

they are mapped in relation to each other as accurately as possible, using compass bearings and distance measurements.

• *To locate the plot(s)*: Flagging tape and painted stakes will help you spot the plots once you are at the site (See Table 1.1). Plots marked with metal stakes, pins, or tags can be relocated with a metal detector.

• *Take photographs* of the plot to have a visual record of changes that occur and assist in relocating the plots. Mark the photo points on your site map.

1.6.5 Describing the site

General site characteristics should be described for a field site even though they may not be identified as the primary factors under investigation. A site description should include the slope, aspect, elevation, longitude and latitude, soil classification (Agriculture Canada Expert Committee on Soil Survey 1987), humus classification (Green et al. 1993), and for sites in British Columbia and some other areas, the appropriate biogeoclimatic ecosystem classification (refer to the B.C. Ministry of Forests regional field guides listed in Appendix C). Topographic grid references are also useful to locate the general area of the site.

Keeping the objectives of the study clearly in focus will help identify other factors that should be documented in the site description because they might affect the outcome of the study. For example, the percent cover of major non-tree species that commonly invade to sites following disturbance should be listed if their presence could influence the results of your experiment. Soil profile details could be included if the experiment would benefit from this information. If the site has been harvested, the degree of soil disturbance should be quantified and

TABLE 1.1 *Installing and marking the research plots*

Stakes	
Weight:	This factor is critical (unless few stakes are needed) if the site is inaccessible and materials have to be carried a long way. In rocky ground, thinner stakes are easier to install. On steep slopes, stakes usually get pushed over in the winter, especially where there is a lot of snow and/or vegetation. Use strong, slim stakes and pound them far into the ground to reduce this problem.
Visibility:	Stakes should be taller than the tallest understorey vegetation, but short enough that you can reach the top to pound it in. Allow enough length to compensate for the amount that is pounded into the ground. Ensure the stakes are clearly visible by painting the tops bright, contrasting colours (white, fluorescent pink, orange, or blue; not yellow, green, or dull or dark colours).
Wood:	*Pros:* relatively lightweight; broader surface more visible when painted; easy to attach labels; moderate cost. *Cons:* bulky; eventually rot; can split and break; may be harder to pound into the ground; greater surface area, more easily pushed over by snow.
Steel reinforcing bar (rebar):	Bend the ends of rebar stakes to prevent injury to people and animals. *Pros:* compact, long-lasting (but rust); relatively cheap; easy to pound into even rocky ground; can be relocated with a metal detector. *Cons:* heavy; not very visible even when painted, difficult when rusty; harder to attach labels; <1 cm diameter can bend fairly easily; larger diameters (>1 cm) are too heavy (except for short stakes).
Aluminum:	Use either conduit or Y-beam. *Pros:* lightweight; visible when unpainted; strong (doesn't bend easily), won't corrode, can engrave plot information directly on so don't have to attach separate labels; can be relocated with a metal detector. *Cons:* expensive (3–4 times cost of rebar).

TABLE 1.1 *Continued*

Installation

Pound stakes until they are as steady as possible. Carpenters' hammers (unless they have metal handles) break too easily. A short-handled 2 lb. (0.9 kg) sledge hammer is ideal because the handle is stronger and the head has a broader surface area. If topofil (hipchain) is used to measure distances, remove the thread after measurement because it can entrap birds and other small animals and kill them.

To form square plots:	Use one rope to measure the length of two sides of the plot (marking the middle), and a second rope for the diagonal length. A tape measure can be used instead if it is long enough. Install the first stake. Measure the distance to the diagonally opposite stake and install. Measure the length of a side towards a third corner from each of the first two stakes. Where these meet, install a third stake. Repeat the last two steps to locate the fourth stake. Ropes with loops on the ends (one the diagonal length and the other the length of two sides with the middle marked) or two flexible fibreglass tapes can be used to make the measurements.
For circular plots:	Install a single stake in the middle of the plot. Use a rope the length of the plot radius to measure from the centre stake to the plot boundaries and mark with flagging tape.

Labelling

Labels are essential if there is more than one plot in the installation. Identify the plot with a number code and other pertinent information. (See also Section 1.5.1.)

Washers:	Stamp large washers (3.5 cm diameter with 1.5 cm hole) with plot numbers using a die set, then slip them over the rebar stakes. *Pros:* easy to use. *Cons:* eventually rust so much you cannot read the numbers; work their way into the ground and must be excavated; can slip off and be lost if the stake is pushed over in the winter.
Aluminum sheets:	Can be wired, nailed, or stapled onto wooden stakes, or folded around rebar stakes and stapled. *Pros:* easy to engrave; won't corrode; easy to see; can attach them to the tops of stakes (no excavating); easy to mold to stake. *Cons:* easily ripped by animals or vegetation rubbing in winter, etc.; sharp edges unless each edge is bent.
Plastic or metal tags:	Can be purchased from engineering or survey equipment suppliers either pre-numbered or blank. Some suppliers will engrave custom numbers on blanks. Pre-numbered plastic livestock ear tags are also available from agricultural suppliers. Plastic tags come in different colours but may break or fade over time. Metal tags are more durable but less visible. Use coated wire to attach tags to plot stakes, trees, etc.
Flagging tape:	If resampling frequently (e.g., every 1 or 2 years or less), use plastic flagging tape on stakes. Use the most durable winter-weight flagging; although more expensive, it lasts much longer. Use fluorescent pink, orange, etc. (same as for paint) for the best visibility. A long tail of tape moving in the wind will catch the eye better than many short pieces and wrap-arounds. Biodegradable tape is not recommended as it is almost impossible to see. If sampling is infrequent, don't bother with flagging; it is not very durable, and animals chew on it. Rely on painted stakes, photos, and good site maps instead. Felt pen on flagging tape is OK for temporary labelling purposes (about one year), but not for long term.

documented using the methods prescribed in the *Forest Practices Code Soil Conservation Surveys Guidebook* (1997) (Appendix C).

When a stand is present on the site, then the description should include an estimate of tree species composition based on basal area. This can be done using variable-radius sample plots. The basal area factor of the prism or relaskop and species of "in" trees are used to estimate species-specific basal area (British Columbia Ministry of Forests 1992). Relaskops are used most commonly, but a set of prisms works as well and is less expensive (about $40 instead of $1400 for a relaskop).

Environmental conditions, such as relative humidity, rainfall, hourly temperature averages, and daily maximum and minimum temperatures, can be monitored using on-site dataloggers. Note that climatic data collected from standard weather stations may not be sufficient to accurately document factors that affect flowering, pollen dispersal, cone opening, and seed maturity at the stand level. Weather variables monitored several metres above the ground may not reflect the conditions within the crown, or on the north and south sides of a tree. In some cases it may be useful to establish correlations to capture these relationships. For further discussion on these and related topics, refer to Section 1.5, Data management, and Section 2.2, Designing an environmental monitoring program.

1.6.6 Site index

The site index is commonly used in forestry to measure site productivity. The site index is the average height of top height trees (unsuppressed dominant or codominant trees) measured at breast height age 50. The more productive the site, the higher the site index. Site index can be obtained in at least two ways: from tree measurements or from the site series. To obtain accurate tree-based estimates of site index, good top height trees should be present in the plot. A description of what constitutes good top height trees can be found in Forest Productivity Councils of British Columbia (1996) and Soderberg and Nigh (1994). These publications also detail the sampling protocol. The total height of the tree and its breast height age are required to estimate site index. This information and the name of the tree species can be entered into the SiteTools software (available from Research Branch, B.C. Ministry of Forests) to obtain an estimate of site index.

When good top height trees are not present on the site, the site index can be approximated through a site series–site index correlation, by which a site index is estimated from the site series present. This method is generally less accurate than the tree-based estimates, and should not be used if good top height trees can be found. Site series–site index correlations are not yet widely available, but may be found in the Ministry of Forests field guides for Nelson (Braumandl and Curran 1992), Prince Rupert (Banner et al. 1993), and Vancouver (Green and Klinka 1994), and in the scientific literature (Green et al. 1989; Klinka and Carter 1990; Carter and Klinka 1992; Wang et al. 1992; Kayahara et al. 1993; Wang et al. 1994). First approximation provincial correlations are available in draft form (Meidinger and Martin [1997]).

1.7 Analyzing and Interpreting the Data

The great tragedy of Science:
the slaying of a beautiful hypothesis by an ugly fact.
(T.H. Huxley)

For many researchers, the most enjoyable (and challenging) part of a study occurs after the data have been acquired and entered into the database. At the data analysis and interpretation stage (Stage III) the relevance of research results must be recognized and articulated. If the project has been well planned (including site selection, experimental design, and analyses), this stage is usually straightforward. However, unexpected things happen, and you may need to manipulate and analyze the data in ways not initially planned. For example, look for confounding factors that may be influencing your results, or try grouping your data differently and reanalyzing.

The value of reanalysis is best demonstrated using an example (D. Coopersmith, pers. comm., 1997). It also demonstrates how a detailed site description can later be used for other purposes.

Recently, an analysis was performed on permanent sample plots at the Aleza Lake Research Forest in north-central British Columbia (lat 54°06'N, long

122°15'W). The stand had been logged in the 1940s using diameter-limit selection (all trees larger than 40 cm were taken, and smaller trees were left). The site was a very productive moisture-receiving site at the base of a long slope. It was also micromounded, probably from previous windfall events in the stand. Some of the spruce were as old as 200 years, so it was probably 300 or more years since this site had been burned. An initial examination of the tree data showed that basal area and volumes had not increased since the last evaluation in 1986. This was surprising because some very large spruce and subalpine fir appeared to be growing very vigorously on the site.

A second analysis was performed. This time the trees were separated into two classes: those growing in the wetter hollows of the micromounds (characterized by *Equisetum*); and those growing on the drier mounds of the site. The results of this reanalysis were dramatic: all trees in the hollows showed little or no growth (in fact, large numbers had died since the last evaluation), showing that trees on these microsites had not contributed anything to basal area and volume in the stand, while those on the higher microsites were still growing vigorously and adding significant additional growth. By not differentiating between these two microsite types, much of the story of these plots was lost in the "noise."

Researchers should consult a statistician before embarking on any of the more elaborate statistical analysis methods to ensure the proper application of the techniques.

1.8 Administration of the Research Site

Field research must always be undertaken with the knowledge and approval of the land owner and the local land manager. In British Columbia, researchers wishing to locate research sites on provincial Crown land must follow the regulations and guidelines set by the B.C. Ministry of Forests and other agencies. The steps outlined in this section are specific for research sites in British Columbia, but are similar to requirements in other areas. Ensure that you contact the agencies with jurisdiction over the area you have chosen and that you know and follow the appropriate regulations.

1.8.1 Obtaining site approvals

For sites in British Columbia, researchers are responsible for obtaining the B.C. Ministry of Forests district manager's agreement for the location and purpose of the research. The district manager will want to ensure that the project can be accommodated within the objectives of the management plan for the site. Researchers must adhere to the Forest Practices Code of British Columbia; for silvicultural system and natural regeneration trials, this may require amendment of silviculture plans and cutting permits. Plans normally must be filed at least 1 year in advance with the district office. For trials not affecting silviculture prescriptions, the district manager must be notified a minimum of 3 months before the proposed research work.

Before beginning any study on developed lands, check with the local offices of major utilities to ensure that site activities will not disrupt water, electrical, or gas services in the area. Field personnel should know the name and telephone number of the appropriate utility companies to contact in case of emergency.

1.8.2 Registering field installations

Some regional offices maintain a list of the objectives and locations of all known research plots within the region. Researchers establishing research plots or installations on provincial Crown land must convey the site location and other pertinent information to the regional research manager, as well as to the relevant district managers.

A protocol for plot registration and map notation has now been established for all permanent sample plots (PSP) by the B.C. Ministry of Forests for the forest inventory mapping system (FAMAP). Map notations are made on all mylars furnished to the forest districts. When a district is proposing a stand treatment, such as thinning or fertilization, all district mylars are checked for the area of the proposed treatment. This is how the forest district avoids treating well sites, archaeological sites, and research plots.

There is a standard procedure for getting information entered on the map mylars, such as a harvesting tenure or an experimental project (EP), usually by providing a sketch map and documentation. Note, however, that the researcher is responsible for keeping track of sub-EPs in the permanent research file.

1.8.3 Security

For security purposes, the location of research sites must be on file with the applicable district, regional, and licensee offices. Installations that will be repeatedly visited (i.e., more than once) should be registered on forest cover maps as either a map notation (coded on the forest cover map to notify users that an activity is occurring there) or a map reserve (to reserve the site from harvest within a specified time period). Map notations or reserves can be critical in saving a site from disturbance or inadvertent damage.

Experimental plots benefit greatly from having signs posted on the site. This will keep out most of the public. At the minimum, the sign should state "Research Site, Do Not Disturb. If you would like more information, please contact the nearest district office." Locate valuable equipment such as meteorological stations so they are not visible from the road; this will lessen the possibility of equipment being vandalized or used for target practice.

1.8.4 Safety

Ensure that you know the radio and check-in protocols for the district you are working in (see B.C. Ministry of Forests Research Branch Operating Policies and Procedures).

Use radio or cellphone communication when possible. Radios are essential on active logging roads. The forest district office or logging company can supply you with radio frequencies, but they are also usually posted at the beginning of the road. The appropriate frequencies can be programmed into radios by staff of the B.C. Ministry of Forests Technical and Administrative Services Branch (for ministry employees) or at most radio shops. In the field, call your location frequently and monitor the location of logging trucks so you can pull over well before you meet them. Logging trucks always have the right-of-way. Always drive with your headlights on when on logging roads.

Report your destination and return time before any field trip, and check in during the day so that someone knows where you are and your next stop. B.C. Ministry of Forests policy advises against working alone in the field and strongly discourages the practice. This policy relates specifically to situations in which an employee is working in a remote area off paved roads, and may be unable to call for help if injured, or is unlikely to be discovered by passers-by. If working alone is necessary, you should know and closely adhere to policy guidelines of the forest management agency, your employer, and your local authority.

In British Columbia, the safety of crews working in the field is governed by Workers' Compensation Board (WCB) regulations and Industrial Health and Safety and Occupational First Aid Regulations. Ensure that you are aware of and comply with all the requirements—only a few are highlighted here. Regulations stipulate that a Level I first aid kit and someone with appropriate first aid training must be on-site. Specific written procedures for transporting injured workers must be developed and be present at the field site before operations begin.

1.8.5 Using registered seeds

In British Columbia, only registered seeds may be used for reforestation on Crown land (Forest Practices Code, Silviculture Practices Regulation, Sec. 2(1)(a)). This regulation also applies to forestry research trials if the seeds will be planted on Crown land. Refer to the *Forest Practices Code Guidebook: Seed and Vegetative Material* or consult with district staff for current seed use guidelines. The Seedling Planning and Registry (SPAR) system can identify available registered seed sources. Contact the district office for assistance with SPAR and other Code-related matters. (See Appendix C for resources.)

1.8.6 Making seed collections

If the seedlings resulting from individual seed collections will not be planted on Crown land, the use of registered seeds is not required. However, if you plan to collect your own seeds, you must obtain a cone collection permit from the district office in which the collections will be made. B.C. Ministry of Forests researchers are not required to have a permit, but they are still encouraged to inform district staff of their intention to collect cones.

Anyone making seed collections is responsible for rigorously adhering to all precautions restricting the use of climbing gear and collection equipment. In British Columbia, aerial operations are subject to WCB regulations, the helicopter company must be certified, and the pilots appropriately qualified for making aerial collections. Refer to WCB regulations, Eremko et al. (1989), and Camenzind (1990) for

additional information on the safety aspects of tree seed collection in British Columbia.

1.9 Ecosystem Management

From time to time, it is beneficial for researchers to stand back and view their research in the larger context in which studies are conducted. By viewing studies of tree seed biology in the broader perspective of ecosystem management needs, research results can have a greater impact and enhanced value to society.

Ecosystem management is a scientifically based land and resource management system that integrates ecological capabilities with social values and economic relations to help sustain ecosystem integrity and use over the long term. In recent years, the term *adaptive management* has been used to describe a modified approach to managing ecosystems. One of the main distinctions of adaptive management is that it emphasizes learning through conscious experimentation, monitoring, and adjustment (U.S. Dep. Agric. For. Serv. 1996b).

The goal of adaptive management is to create and maintain sustainable ecosystems. To achieve the goal of sustainability requires that we integrate both the human societal and economic needs and ecological processes. This concept may be visualized by viewing the needs of society and the earth's ecological capacity as separate spheres (Figure 1.3). Knowledge and understanding draw these circles closer together. Opportunities for sustainability increase when we manage so that these spheres can overlap.

Information is a primary resource, and as researchers, it is our major contribution; the success of adaptive ecosystem management depends on the generation and transfer of our scientific knowledge (Bormann, Brookes, et al. 1994). Monitoring and research must be integrated with decision-making processes to continually improve the scientific basis of ecosystem management (Jensen and Everett 1994). Thus, it is critical that we allocate our efforts to bridge this interface between science and management. In a topical article, Larsen et al. (1997) defined 10 principles of ecosystem management which provide useful guidance to ecosystem researchers to make their research projects relevant to management needs for information. Not all research projects will be able to strictly adhere to these principles, but they provide a useful reminder of context for natural resource studies.

1. **Management and research must deal with large landscapes.** The cumulative effects of processes that typically function at smaller scales, such as stand-level silvicultural treatments, can be observed only if we step back to take a wide-angle view of the forest. Some important processes, such as patterns of forest distribution or natural disturbance, can be observed only at the landscape scale.

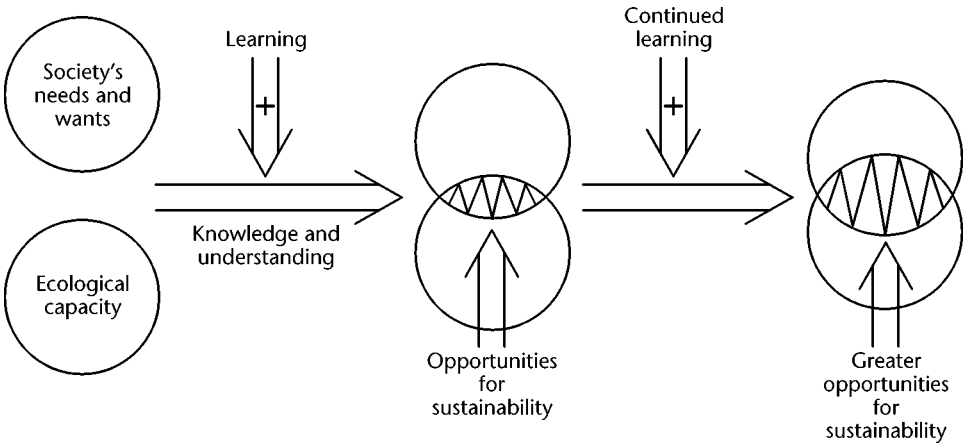


FIGURE 1.3 *Sustainability can only be achieved when the needs of society and the potential capacity of the earth we live in overlap. Learning draws these circles closer together and increases our opportunities and options for sustainability. (Adapted from Bormann, Cunningham, et al. 1994.)*

2. **We must be concerned with long time frames.** Just as the extent, structure, and condition of today's forests have been determined by harvesting practices that took place a century ago, so the impact of the current management activities will persist at least a century into the future.

3. **We must consider both where and when we create a disturbance.** Important spatial and temporal components are associated with any forest management activity or any natural disturbance. If, for example, our management activities will disturb large areas in a given landscape over the next century, it makes a difference whether the affected areas are contiguous or dispersed, and whether the disturbance occurs in a single year or is spread over the full century.

4. **We have enough scientific knowledge to start managing ecosystems,** but we will never fully understand all aspects of forest ecosystems. We know a great deal about some parts of forest ecosystems and at least a little about most parts; a prudent approach is to begin by using the best science we have available now, while we continue with our research.

5. **We must synthesize the results of research that address many different ecosystem attributes and processes.** We must combine what we know about ecosystem components and ecosystem processes to arrive at a more complete understanding of how ecosystems work and how they respond to disturbance. Synthesis also serves to identify the major gaps in our knowledge.

6. **The complexity associated with ecosystem management is so great that we must employ mathematical models.** Tracking details, measuring interactions and trade-offs, dealing with long time frames, dealing simultaneously with many species, and mapping the results—all require the use of computer models.

7. **We must facilitate cooperation and collaboration.** The complexity of forest ecosystems requires the attention of teams of scientists and managers representing a wide range of expertise.

8. **Researchers must share sites so that they can integrate their findings and investigate change in each ecosystem component over many different spatial and temporal scales.** Agencies must make long-term commitments to maintain research sites as well as to fund basic site measurements. The marginal cost of additional projects is quite low as long as a base level of measurements exists.

9. **We must simultaneously focus our collaborative research efforts on real landscapes.** We will increase our understanding of the interactions and trade-offs only when experts from many fields apply their collective wisdom to the same piece of land over the same time frame. Purely theoretical approaches to ecosystem management research have great merit, but ultimately the evaluation must be in the field.

10. **We must remember that people are part of the ecosystem.** Human activity has left an indelible mark on our forest resources, and ultimately, it is people who decide which forest practices are acceptable. Our role as scientists and practitioners must be to: (a) identify and discourage those activities that will likely cause short-term or long-term ecosystem degradation, (b) clarify the trade-offs among many acceptable management alternatives, and (c) identify and encourage the alternatives that will most likely produce the desired outcomes.

1.10 Summary

A long-term experiment whose sole sponsor has left, died, or lost interest is a sad orphan, and adoption is seldom quite successful.
(Dyke 1988)

Planning constitutes the major activity associated with field studies, and may even take longer than the study itself. Care is needed in defining the experimental protocol, data management, and reporting routine. Good plans are especially critical when the main investigators are not readily available at all times during plot establishment and site selection.

Flexibility is also required, and possible modifications should be considered even while the study is being conceptualized. The plan must try to anticipate some level of uncertainty and be flexible enough to cover unexpected conditions in the field. Change is inevitable, and consideration of alternative approaches during the design stage will help to focus the planning effort and secure long-term success of the project.

Field experiments require sustained commitment by the scientific staff so that the study will reach its full potential. Sustained commitment by funding organizations is also essential to maintain stability. Finally, the information needs urgently required by natural resource managers necessitates that the results of field studies reach the end user as quickly and as accurately as possible.

The role of a scientist in the ecosystem management model is to provide information for the decision-making process. Such information helps to identify the current status of an ecosystem as well as potential options for addressing the social, physical, economic, and biological issues (Haynes et al. [technical editors] 1996). This information helps clarify feasible limits, options within the limits, consequences of those options, and trade-offs between options. It is the role of the decision-maker to choose among options; it is not the role of science. The challenge for resource managers is to balance biological science with social science and with the philosophical views of how society values renewable and nonrenewable natural resources (Haynes et al. [technical editors] 1996).

SECTION 2 DESIGNING AN ENVIRONMENTAL MONITORING PROGRAM

Every raincloud, however fleeting, leaves its mark,
not only on trees and flowers whose pulses are quickened,
and on the replenished streams and lakes,
but also on the rocks are its marks engraved …
(John Muir "Gentle Wilderness, the Sierra Nevada")

2.1 Background

Environmental and site factors influence the production, dispersal, survival, longevity, and germination of tree seeds. Researchers must have a general understanding of the effects of various environmental factors to select the most suitable location, time frame, experimental techniques, and types of sensors for field studies. The nature of these factors and the overall objectives of the study will also determine which environmental variables should be measured, and how frequently.

Environmental variables such as temperature and precipitation may be considered in the context of long-term average conditions, as ranges and extremes (climate), or as day-to-day conditions (weather). Furthermore, environmental variables can be viewed at three scales: macro (or regional) weather, site weather, and tree weather. The complexity involved in obtaining data increases as we go from macroclimate to tree weather.

Macroclimate and synoptic weather conditions can be obtained from the nearest Environment Canada or other government-operated weather stations, and may be adjusted for the elevation of the site of interest. Usually, climate data are summarized as monthly or annual values and include average, maximum, minimum, and extreme values for temperature, total precipitation, and derived data, such as degree-days and frost-free period. Wind speed and direction are available for a few locations. For some parts of British Columbia, annual temperature and precipitation summaries by subzone can be obtained from the biogeoclimatic ecosystem classification database (see Appendix C).

Site climate and site weather conditions involve on-site measurements of air and soil temperature, precipitation, humidity, wind speed and direction, solar radiation, and soil moisture. Monitoring usually requires an electronic datalogging system. Weather data may only be needed for a short time during an event of interest (e.g., pollen release); in this case, you may need hourly rather than daily summaries. However, to characterize the climate (averages and variation), 5–10 years of data collection are required. These data should be referenced to the nearest long-term weather stations to determine how different the period being measured may be from the "normal."

Tree weather describes conditions in cones or flowers, or beside germinating seeds. Small, delicate sensors, such as thermocouples, are usually required to make these measurements. Variables of interest in regard to tree weather are temperature, radiation balance, and soil moisture (for germination). The data can be used to develop physically based models or regression models of tree conditions as a function of site conditions.

2.2 Designing an Environmental Monitoring Program

In designing an environmental monitoring program for a research site, you must first decide which variables you need to measure. For field germination studies, near-surface (0–5 cm depth) soil temperature and soil moisture are the important variables. However, soil temperature and moisture will be affected by a variety of other environmental variables. Soil temperature, for example, depends on soil moisture, solar radiation, wind speed, air temperature, soil texture, and surface colour. On the other hand, surface soil moisture depends on rainfall, solar radiation, evaporation, vegetation cover (transpiration), soil texture, and soil temperature. The humidity of the air and solar radiation can critically affect the initial establishment of germinants through its effects on soil evaporation and plant transpiration. Humidity also affects seed production through its effects on pollination. Slope and aspect affect temperature and moisture because they influence the solar radiation and rainfall reaching the surface. Wind is of interest primarily for studies of seed dispersal (see Section 4).

The frequency of environmental measurements will vary depending on the type of measurement. Light and temperature can vary rapidly and thus require frequent monitoring. Relatively stable site factors, such as soil type, soil pH, presence and type of duff layer, biogeoclimatic zone, elevation, slope, and aspect, may need only to be measured once.

Researchers sometimes rely on environmental data from the nearest weather station to provide data such as rainfall and daily minimum and maximum temperatures, but if the microclimate of the site is significantly different from that of the weather station it is advantageous to set up a small weather station at the site (Figure 2.1). Dataloggers can be used to continuously record a variety of environmental variables (Figure 2.2). Spittlehouse (1989) provides guidance on using dataloggers in the field and the accuracy that can be expected from such measurements. While it is tempting to collect large amounts of weather data on the assumption that somehow they will be useful, a few days of manual measurements under a range of weather conditions may be just as effective as installing electronic dataloggers on the site. The disadvantage of manual sampling, however, is that you may be able to demonstrate differences between treatments only when they are large.

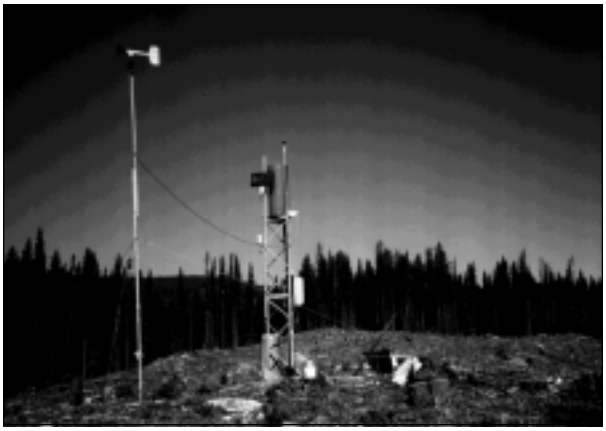


FIGURE 2.1 *Climate station with wind direction and wind speed sensor, a rain gauge (lower right), and tower with solar radiation, air temperature, and humidity sensors. A robust tower is required at this site to support the large precipitation gauge used for winter snowfall measurements.*

Whatever the means of recording data, the complexity of environmental factors and their interactions necessitates careful planning of all field measurements. Four steps to developing an environmental monitoring program are illustrated here using an example of a study to determine conditions that initiate flowering.

1. **Why do you need environmental data?**
To determine weather conditions that initiate flowering, and their variation from year to year.

2. **What data are needed?**
Air and bud temperatures and solar radiation, from bud initiation through flowering (over 6 months or more). The year-to-year variation could be obtained by monitoring for many years, or by calculating regression equations that are based on weather data. Site weather conditions could be related to the nearest long-term weather station to provide the data that would be needed to drive the model. Ideally, a physically based model of bud/flower temperature as a function of site weather conditions and bud characteristics should be developed to allow portability to other sites with a minimum of calibration. Both methods require 2–3 years of on-site data for development and validation.

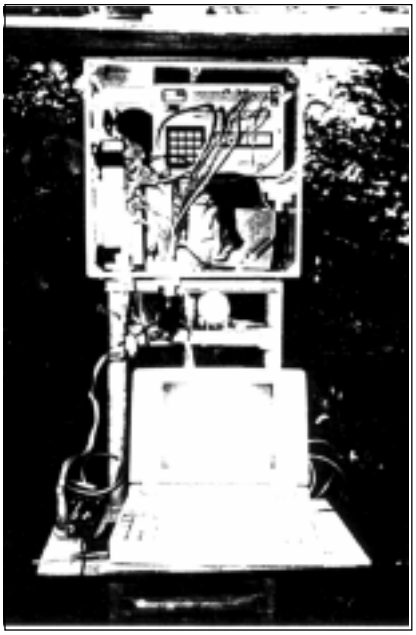


FIGURE 2.2 *Electronic datalogger used to monitor and capture data from a series of environmental sensors. The datalogger is housed in a waterproof box (shown open) and data are routinely retrieved using a portable computer. This datalogger can monitor many sensors at once; less expensive dataloggers are available that will monitor only one sensor.*

3. **What needs to be done to get the data?**
Is continual monitoring necessary, or is a short-term manual measuring program adequate? Fine wire thermocouples are needed in the buds to measure temperature; they are sensitive and inflict minimal damage. The site may need frequent visits to ensure that bud temperature sensors have not been disturbed.

4. **Can the work be done physically and what does it cost?**
Is the site readily accessible, and are equipment and personnel available? A datalogger-based monitoring system would cost $300 to $7000. Installation requires 1–3 days depending on the number and location of sensors, and monthly site visits are required to check equipment and collect data. At least 1 day per month should be allowed per site for data processing and analysis—an important consideration that is often overlooked.

2.3 Methods for Measuring Environmental Factors

2.3.1 Soil temperature

Near-surface soil temperature (0–2 cm depth) can be easily measured, but measurements must be adequately replicated. Soil temperature varies substantially, not only horizontally and vertically, but temporally as well. Individual locations can be averaged by using a series of thermocouples connected in parallel. Dataloggers are a convenient way to monitor the number of sensors required to assess the spatial and temporal variability. The diurnal trend of the near-surface temperature parallels the diurnal course of solar radiation; thus a reasonable approximation of the daily maximum temperature can be obtained by making the measurement about an hour after solar noon. An estimate of solar noon in your area is available on the Internet at http:/www.crhnwscr.noaa.gov/grr/sunlat.htm. The average near-surface soil temperature (during the summer) can be approximated by measuring at about 8 a.m. solar time (4 hours before solar noon). A comparison of treatments with this approach requires that measurements be made under the same weather conditions. This manual sampling method will only be useful for showing differences larger than 5°C and should only be used to give an idea of trends.

Shade can significantly reduce solar radiation, resulting in a corresponding decrease in the near-surface temperature. On the other hand, shade also reduces night-time cooling. When solar radiation is reduced by over 60%, the surface temperature can be approximated by the air temperature at 1.5 m above the ground. For more information on forest soil temperature, see Stathers and Spittlehouse (1990).

2.3.2 Soil moisture

There is no easy way to obtain good soil moisture measurements, and the difficulty increases as one gets closer to the surface. Gravimetric sampling and time-domain reflectometry (TDR) measure soil water content (Hook et al. 1992), but further work is required to develop TDR probes and techniques. TDR requires substantial replication, and although it is usually done manually, it can be automated. Water content can be converted to soil water potential (or tension) using a soil water retention curve obtained

from undisturbed soil samples analyzed by a commercial soil physics laboratory.

Gravimetric sampling is labour intensive and destructive. Some 5–10 replicates at each depth of interest are required. It is best to use a sharpened metal tube to take a soil core of known volume, rather than a grab sample. The sample is sealed in plastic bags, and returned to the laboratory for weighing and drying. Gravimetric samples are presented on either a weight of dry soil or a volumetric basis. The latter is the common approach and can be converted to soil water potential (or tension) using a soil water retention curve.

Gypsum or fibreglass soil moisture blocks can be used to measure soil moisture potential (or tension) in the 0 to -2.5 MPa (25 bars) range; they can be read manually or with a datalogger. Moisture blocks provide relatively coarse resolution, and require testing over several drying cycles before installation. They may have poor contact with substrates such as coarse sandy soil or partially decomposed organic material and cannot be used at soil depths shallower than 5 cm.

Tensiometers measure soil water potential in the 0 to -0.08 MPa (0.8 bars) range. They are usually read manually but can be automated. As with moisture blocks, they cannot be used at depths shallower than 5 cm. Soil water potential can also be obtained by equilibrating soil samples with the air or filter paper in a sealed container, then measuring the humidity of the air or filter paper. *In situ* measurements of soil water potential using soil psychrometers or hygrometers is extremely difficult, particularly in the top 15 cm of the soil. Further information on measuring soil moisture can be found in Schmugge et al. (1980).

2.3.3 Solar radiation (light)

Three ranges of the radiation spectrum are usually considered when assessing the light regime at the earth's surface: ultraviolet radiation from 290 to 400 nm, photosynthetically active radiation (PAR) from 400 to 700 nm, and solar radiation from 300 to 3000 nm. Radiation above 3000 nm is called longwave or thermal radiation. Different sensors are required to measure each of these bands of energy. There is a good correlation between the energy in each band both above and below the canopy (Yang et al. 1993; Alados et al. 1996).

Radiant flux density is the energy in the light emitted, transmitted, or received per unit area (W/m^2). Irradiance is the radiant flux density incident on a surface; emittance is the radiant flux density emitted by a surface.

The units and the instruments used for light measurement will depend upon the intent of the study. Some radiometers (for example the LI-COR model LI-189 radiometer) can be fitted with a variety of sensors to measure irradiance. A quantum sensor is used to measure photosynthetic photon flux density (PPFD) or PAR. A pyranometer (or radiometric sensor) is used to measure solar radiation. Photometric measurements using illumination units (lux or footcandles) should not be used in plant studies. Plants do not respond to the light spectrum in the same way as the human eye, so such measurements have no value unless the characteristics of the light source are precisely known (Salisbury and Ross 1992). PAR is usually the radiation measurement made when assessing physiological responses such as plant productivity (although other wavelengths may have specific photomorphogenic effects such as the induction of flowering or cold hardiness). PAR is commonly measured in units of $\mu mol \cdot m^{-2} \cdot s^{-1}$. Special sensors are required to measure ultraviolet radiation, and various filters are available that modify sensor output to match the biological response of tissue. Longwave radiation sensors are not easy to use, so longwave estimates are usually obtained by subtraction of solar radiation from total radiation measurements (see Black et al. 1991).

The controlling influence of vegetation on the light regime will render a shaded surface more moist and cool than a bare surface. The amount of direct cover over the study area, the distance from the edge of openings, and the aspect of the edge will influence the light regime in openings. These influences can be estimated from measurements of above-canopy light and the amount of cover. When measuring irradiance under a plant canopy with uneven light levels, reasonable averages can be obtained by moving a small sensor repeatedly along a track (Figure 2.3), by using a long linear sensor, or by using many spot sensors. It is generally best to spend some time generating radiation interception curves with an intensive measurement program over a short period. These curves are used with continuously monitored above-canopy

FIGURE 2.3 *Automatic tram system that moves back and forth over a 50 m span to determine the variation in short- and longwave radiation, and surface and air temperature under a forest canopy. The system is controlled by the datalogger in the tube hanging on the end. (System designed by R. Adams, B.C. Ministry of Forests.)*

radiation to give below-canopy data through the year. The variability or patchiness of the canopy may indicate that there is a range of light environments that must be quantified separately. Radiation rapidly decreases as canopy cover increases. The interception curve is of the form

$$I = I_o e^{(-KC)},$$

where:

I = the radiation at the forest floor,
I_o = the radiation above the canopy,
C = percent canopy cover (range 0–100), and
K = an extinction coefficient (range 0.02–0.04).

Shaded and sunny areas of small openings and clear-cut edges can be determined using the formulae for length and direction of tree shadows at different times of the day and year (Sit 1992a).

Forest canopies change the quality as well as the intensity of light reaching the forest floor (Vezina and Boulter 1966; Atzet and Waring 1970; Ross et al. 1986; Messier et al. 1989). Figure 2.4 illustrates how the spectral distribution is changed due to plant foliage differentially absorbing and reflecting the various wavelengths. The relatively thick needles of coniferous trees transmit very little radiation and most radiation reaching the forest floor passes between needles and other gaps in the canopy. Consequently, the change in the shape of the spectrum is not as great as in hardwoods where more radiation passes through the thinner leaves. The biggest change is in the increase in the ratio of red (640–680 nm) to far-red (680–760 nm). It changes from 1.1–1.3 under clear skies above the canopy to 0.08–0.28 under hardwood forest canopies, and to 0.6–1.0 under coniferous canopies.

Light quality—the incident light spectrum—affects the germination of many conifer seeds (Section 7.1.2) and the production of female cones (Durzan et al. 1979). For measurement of irradiances under forest canopies, see Black et al. (1991) and Yang et al. (1993). The measurement of the total light spectrum

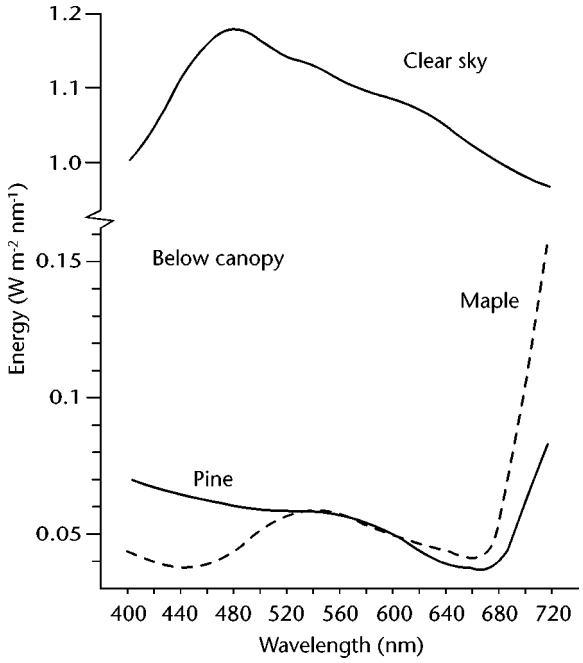


FIGURE 2.4 *Influence of forest canopy on the intensity and spectral distribution of solar radiation reaching the forest floor. The upper panel shows the incident radiation from a clear sky during the middle of the day. The lower panel (note the difference in scale) indicates that pine and maple canopies have greater absorption in the middle range (400–700 nm) than in the near infrared range (700–750 nm). (Based on data in Federer and Tanner 1966 and Vezina and Boulter 1966.)*

at a site requires a portable spectroradiometer. Both Atzet and Waring (1970) and Floyd et al. (1978) conducted spectroradiometric analyses to determine the light-filtering capacity of coniferous forests. However, the changes in light quality can simply be measured with a portable red:far-red meter, since most of the canopy effects are due to the canopy cover shifting the ratio of red to far-red light.

2.3.4 Wind speed and wind direction

Wind, acting either directly or by wind-induced vibration, plays a major role in the distribution of pollen and seeds. Seeds of some species are very responsive to updrafts or vertical air movements (see Section 4.1.3). Wind speed is a vector quantity with attributes of direction and magnitude, although only the horizontal component is usually measured. Cup or propeller anemometers are generally used to monitor wind speed. They can be connected to a datalogger or have their own display. Many ane-mometers come with a wind direction indicator. Topography and vegetation cover affect wind speed and direction and care must be taken in locating the anemometer and wind vane. The sensors should be located 2–10 m above vegetation canopy and away from clearcut edges. Robust anemometers usually have stall speeds of 0.5 m s^{-1} or greater. Low stall speed anemometers are required if you are interested in conditions at the edge of a clearing, in a small opening, or below the canopy. Hot-wire anemometers (commercial or home-made) can be used to measure wind flow around cones and flowers, but they are delicate and easily damaged. They can be monitored manually or with a datalogger. A discussion of wind dynamics and instrumentation can be found in Pearcy et al. (1989).

2.3.5 Precipitation

Rainfall can be reliably measured with tipping-bucket or storage gauge. The gauge should be located in an opening where a line projected from the top of the gauge to the top of the surrounding vegetation has an angle of no more than 45° to minimize any shading effects. Snowfall cannot be measured with a standard tipping-bucket gauge. Although gauges that melt the snow and can be monitored with a datalogger are available, they are expensive. A low-power, reliable sensor that measures the depth of snow on the ground can be used in conjunction with a datalogger.

2.3.6 Air temperature and humidity

A hygrothermograph in an instrument shelter (Stevenson Screen) located on the study site can record air temperature and relative humidity for up to a month before the chart requires changing. Electronic temperature and humidity sensors can be placed inside the Stevenson Screen. Smaller shields can be built or are available commercially, but some commercial varieties overheat under low wind speeds. Spot measurements can be made using aspirated or sling psychrometers.

2.3.7 Plant temperature

Obtaining temperature measurement in cones and buds requires extremely small sensors. Thermocouples (Figure 2.5) are the best option, being more robust and easier to make than thermistor or platinum resistance sensors. They are best monitored with a datalogger. Surface temperature can be measured using an infrared thermometer with a narrow field of view.

2.3.8 Canopy cover

Canopy cover is the environmental factor most immediately affected by forest harvesting activities and by silvicultural practices (see Section 8). It significantly affects the microclimate of a site, influencing the solar radiation, air and soil temperatures, wind speed, humidity and rainfall experienced at the ground (Hanley 1978). Canopy cover is also important because the position of vegetation within the canopy is used as a criterion for the relative dominance of individuals within a plant community (Section 3.2.3).

FIGURE 2.5 *A fine wire thermocouple is used to measure the temperature inside the leader of a young spruce tree. The thermocouple is monitored with a datalogger, as are the accompanying environmental sensors. The same technique can be used to measure cone temperature.*

Canopy cover is often expressed as a percentage value, usually by species, growth form, or canopy stratum; in a dense or multilayered community, total vegetative cover may exceed 100%. The method chosen to measure canopy cover depends on the available technology and the type of site; some methods are suitable for low herbaceous vegetation or clearcut areas, while others are designed for forested areas. Bunnell and Vales (1990) present a comparison of different methods of measuring forest canopy cover.

Many researchers obtain percentage cover of different species and canopy layers with the visual-estimation technique (Mueller-Dombois and Ellenberg 1974). Cover can be estimated to the nearest percentage point or to the nearest 5th or 10th percentile. Cover estimates may be restricted to the plots being studied for germination or other responses, or may be used for more general descriptive purposes (such as describing the study site, Section 1.6). The visual-estimation method is especially suitable for grasslands or clearcuts, because of the low profile of the vegetation. Plot size for cover estimation averages about 1.0 m^2 (either circular or square), but may be smaller when working in exclusively herbaceous vegetation, or larger when working with tall shrubs and trees. A good guideline for plot size is that plot diameter should be approximately equal to the height of the vegetation being described.

Visual estimates are subject to personal bias, thus human error will introduce variability into the data (Bunnell and Vales 1990). This can be checked and corrected (calibrated) by other people working on the same project. It is also useful to have examples of how different spatial arrangements affect one's perception of canopy cover. Figure 2.6, for example, compares different spatial arrangements of 50% canopy cover. These kinds of comparisons are especially useful when observers are not experienced in canopy estimation methods.

Canopy cover can be measured more objectively using a line-intercept method, which is suitable for woody plants, shrubs, and trees (Chambers and Brown 1983; Habitat Monitoring Committee 1996). A line or tape measure is stretched tightly across the vegetation between two stakes. The best sampling procedure is the stratified-random sample using a baseline and perpendicular transects (see Chambers and Brown 1983 for a sample layout). The length of the canopy intercept of each species along the line is measured from the tape or with a ruler. If the canopies overlap in layered vegetation, it may be desirable to measure each height layer separately. Transect lines should be 10–100 m in length. Many short lines are generally preferred to a few long lines; 5–10 transects are usually required for an adequate sample. Several cover values can be calculated:

$$\text{percent cover for each transect by species} = \frac{\text{length intercepted by a species}}{\text{transect length}} \times 100;$$

$$\text{percent cover of a species by sampling unit} = \frac{\text{sum of all transect lengths intercepted}}{\text{total transect length sampled}} \times 100.$$

While this method is generally precise and accurate (Cook and Stubbendieck 1986), it can also be time consuming.

FIGURE 2.6 *Different spatial arrangements comprising 50% canopy cover. Some experience may be needed to estimate different proportions of cover.*

Objective measurements of canopy cover can also be obtained with a point-intercept method (Owensby 1973; Levy and Madden 1993) or point-quadrat method (Chambers and Brown 1983). These methods use a point- or pin-frame, often consisting of 10 pins spaced 5 or 10 cm apart, with pins positioned vertically or at an inclined angle. The frame is positioned randomly within the sampling units or along a transect and a single pin lowered towards the ground. The first strike of any part of the vegetation canopy becomes a "hit." Each "hit" is recorded by species or growth-form (Chambers and Brown 1983). The sample size required for statistical adequacy is usually 100–300 pins. Several cover values can be derived from this information: percentage canopy cover for each species or life-form (Chambers and Brown 1983); percent total canopy cover; and percentage vegetation cover by species. The user should be aware that the line is the sample unit, so it is better to have fewer points per line and more lines, rather than vice versa (Bonham 1989). The frame should be held vertically; if the frame is at an angle, the number of intercepts may increase and overestimate the cover.

In woodland areas, other instruments such as the moosehorn and the spherical densiometer are frequently used to measure tree canopy cover (Lemmon 1957; Bunnell and Vales 1990). The moosehorn is a point-intercept method where the canopy is viewed through a screen and coincidences between vegetation and dots on the screen are tallied. The spherical densiometer has a curved mirror with a grid that reflects the overstorey at a particular point, then provides an estimate of the relative amount of the grid covered by vegetation. At each location, four readings (facing north, east, south, and west) are recorded and averaged.

A canopy analyzer uses measurements from a fisheye optical sensor placed above and below the plant canopy; in this way canopy transmittance data can be used to calculate the leaf area index and the mean leaf inclination angle (Chen et al. 1991; Welles and Norman 1991). The canopy analyzer functions in a variety of sky conditions, with overcast being the best; the instrument can be used in canopies ranging in size from short grasses to forests.

The area of leaf per unit area of ground (leaf area index - LAI) is another measure used to quantify canopy cover. It is measured by sampling the foliage or by using light penetration techniques (Gholz et al. 1976; Smith et al. 1993; Fassnacht et al. 1994; Chen 1996). In the former method, all the foliage in the shrub and herb layers is removed from 5–10 samples of known area (usually 1 m^2). The area of the leaves is then measured using an image analyzer. All the leaves from a tree (or from a representative branch in each whorl) are sampled and leaf area measured with an image analyzer. Trees of different diameter at breast height (dbh) are sampled to produce a dbh/leaf area relationship (or sapwood cross-sectional area/leaf area) which is then used with stand dbh distribution to calculate tree LAI. Leaf area can be determined using light sensors such as the ceptometer and the LAI-2000. Both measure the "effective" leaf area and must be corrected for leaf clumping to get the true leaf area index (Smith et al. 1993; Fassnacht et al. 1994; Chen 1996). These sampling techniques can be used to determine how leaf area changes with height and to calculate foliage area density.

The canopy can be photographed from ground level using a camera fitted with a hemispherical or fisheye lens with a 180° field of view. Film exposure must be standardized (Chen et al. 1991).The resulting photographic negatives, prints, or slides can be digitized, and then analyzed by a computer program to accurately measure canopy cover above the point of measurement. Available computer programs include SOLARCALC (Chazdon and Field 1987), GLI (Canham 1988), SUNSHINE (Smith and Somers 1991), and HEMIPHOT (ter Steege 1993). This photographic method is suitable in herbaceous, scrub, forested, or mixed cover, but has the drawback that considerable office time is required to obtain cover estimates. These same programs also model solar radiation input for the point at which the photograph was taken (see Section 2.3.3), but the cover estimates require fewer assumptions.

2.3.9 Soil variables

Soil nutrient levels are important because they affect seed production, germination, and seedling growth. Three principal methods are used to diagnose nutrient deficiencies: deficiency symptoms, soil chemical analysis, and foliar analysis. (For other methods see Morrison 1974.) Soil chemical analysis has some value for diagnosing site nutrient status on sites where

foliage sampling is impractical. There are major problems with conducting soil chemical analysis in forest soils. Typically, the root zone is not homogeneous, often containing dissimilar horizons that may yield different analytical values. Nutrient standards for forestry soils are not available, and criteria cannot be extrapolated from one kind of soil to another. It can be problematic to integrate these disparate results to determine the nutrient status of the composite soil profile.

The high variability of some soils may require a large number of samples. The most useful routine soil chemical analyses for forest soil fertility in B.C. are likely to be: pH, organic carbon concentration, and total nitrogen concentration (Watts [editor] 1983).

Oxygen is usually a limiting factor only in waterlogged soils, where water may fill pore spaces. Oxygen is difficult to measure in the field, but under suitable conditions, an oxygen electrode may be used. This technique uses glass electrodes that are delicate and easily broken, and is not generally suitable for field studies. It is primarily designed for laboratory studies, but can be set up and operated adjacent to a study site. A key requirement is constant-temperature water, obtained from a thermoregulated circulator or a large-reservoir flow-through system. Some instruments can be configured to determine oxygen indirectly by heating the sample in a stream of inert gas and converting all oxygen-containing gases to carbon monoxide or carbon dioxide. Refer to Pearcy et al. (1989) for further information on such methods.

SECTION 3 NATURAL SEED PRODUCTION

O sweet spontaneous
earth how often have
the
doting

fingers of
prurient philosophers pinched
and
poked

thee
, has the naughty thumb
of science prodded
thy

beauty .
(e.e. cummings)

3.1 Background

The path to the production of a viable seed begins with the growth and development of reproductive buds, continues with pollination and fertilization, and ends with embryo development and seed maturation. Throughout all these developmental stages losses occur for a variety of reasons; losses may be due to environmental factors, or may result from various diseases and animal predators that attack cones and seeds. Researchers investigating tree seed production must be able to assess which and to what extent these factors limit natural seed supplies.

Angiosperms characteristically produce seeds annually, but production can vary considerably from year to year (Table 3.1). Most conifers do not produce collectable crops every year (Table 3.2), a phenomenon called periodicity. Mature cones are produced at intervals ranging from 3 to 10 years, and sometimes as infrequently as every 15 years. Crop yields vary in different years, and in poor crop years, the quality of seeds also tends to be poor (Edwards 1980; Caron and Powell 1989a, 1989b). Depending upon the species, conifer seed production varies in length and complexity of the production cycle (Figure 3.1), reproductive success (due to different sexual mechanisms), and the timing of natural seedfall (Zobel 1979).

The reproductive structures of trees are derived from reproductive buds. The time of initiation of male and female reproductive buds can vary from year to year due to factors such as the relative abundance of seed trees (trees/ha) (Smith et al. 1988) and tree age (Caron and Powell 1989b). Natural seed production is rare in trees younger than 10 years. Generally, the volume of seeds produced increases as the tree ages. Bergsten (1985), however, found no biological differences between mature Scots pine seeds obtained from young stands and those collected from old stands. Environmental conditions, such as temperature, drought (Eis 1973a, 1976), and nutrient availability (Heidmann 1984), affect reproductive bud production. Environmental stresses can reduce the

TABLE 3.1 *Seed production characteristics of hardwoods native to British Columbia. Sources: Schopmeyer (1974); Pojar and MacKinnon (1994); Wyckoff and Zasada [1998]; Zasada et al. [1998]*

Species Common name	Tree type	Flowers (description)	Flowers (month)	Seeds mature (month)	Fruit (description)	Average # seeds/fruit	Interval between crops
Acer macrophyllum bigleaf maple	Monoecious; imperfect flowers	Greenish yellow (3 mm across); numerous in hanging cylindrical cluster; male and female in different parts of crown	Appear with or before leaves (Apr–May)	Ripen Sept–Oct; disperse Oct–Jan	Paired winged seeds (samaras); 3–6 cm long	2 (1 / samara); no endosperm	1 year
Alnus rubra red alder	Monoecious	Drooping reddish male catkins (5–12 cm); female catkins are woody cones (2 cm)	Male, previous fall; female, Feb–May	Ripen Aug–Sept; disperse Aug–spring	Brown cones (2 cm) remain over winter; contain oval, winged nutlets	50–100 seeds / cone no endosperm	4 years
Arbutus menziesii arbutus	Monoecious; perfect flowers	White, urn-shaped (7 mm), in large drooping clusters	Apr–May	Fall	Orange-red berry-like (1 cm), surface finely granular	20 hard, angled seeds + endosperm	1 year
Betula papyrifera paper birch	Monoecious	Male staminate flower and female strobile (2–4 cm); break up at maturity	Male, previous fall; female, Apr–June	Ripen Aug–Sept; disperse Aug–spring	Nutlets with wings broader than body	Numerous no endosperm	2 years
Cornus nuttallii Pacific dogwood	Monoecious; perfect flowers	Small (3 mm) white in tight clusters surrounded by 4–6 white showy bracts (2–7 cm)	Always in spring; often in fall	Ripen late summer/fall	Clusters of bright red "berries" (1 cm); globular or ovoid drupes	1–2 / stone; + endosperm	
Fraxinus latifolia Oregon ash	Dioecious	Small (3 mm) male (yellowish) and female (greenish) flowers in bunched clusters on twigs	Appear before leaves	Late summer/fall	Paddle-shaped, samara (3–5 cm) in large clusters on female trees	1 / samara + endosperm	
Malus fusca Pacific crab apple	Monoecious; perfect flowers	White to pink fragrant blossoms (2 cm); 5–12 in flat-topped clusters	Apr–June	Late fall	Yellow to reddish small (10–15 mm) fleshy pomes	3–5 carpels 1–2 seeds / carpel + endosperm	2–4 years
Populus balsamifera ssp. *balsamifera* balsam poplar	Dioecious	Male and female catkins	Apr	June–July	2-valved capsules; not hairy	Numerous; no endosperm	1 year
Populus balsamifera ssp. *trichocarpa* black cottonwood	Dioecious	Male catkins (2–3 cm) with 40–60 stamens/ flower, female catkins (8–20 cm) with 3 stigmas/ flowers	Appear before leaves (Apr–June)	May–July	Round, green hairy capsules that split into 3 parts; seeds covered with white fluffy hairs	Numerous; no endosperm	1 year

TABLE 3.1 *(Continued)*

Species Common name	Tree type	Flowers (description)	Flowers (month)	Seeds mature (month)	Fruit (description)	Average # seeds/fruit	Interval between crops
Populus tremuloides quaking aspen	Dioecious	Male catkins (2–3 cm); female catkins (4–10 cm)	Apr–May	May–June	Catkins of tiny, greenish capsules covered with cottony down	2–7 / capsule; 77–500 seeds / catkin no endosperm	4–5 years
Prunus emarginata bitter cherry	Monoecious; perfect flowers	5–10 white to pinkish flowers (10–15 cm) in flat-topped cluster	Apr–June	July–Sept	Dark red drupes (1 cm)	1 / drupe	
Quercus garryana Garry oak	Monoecious	Tiny inconspicuous flowers; male, in hanging catkins; female, single or in small clusters	Feb–May	Aug–Dec	Acorns (2–3 cm) in shallow, scaly cups	1 / acorn; cotyledons only, no endosperm	2–3 years
Rhamnus purshiana cascara	Monoecious	Loose clusters of 5–12 tiny, yellowish-green flowers	Apr–July	July–Sept	Purplish-black, round berrylike drupe	2–3 nutlike seeds / drupe + endosperm	Good crops alternate with poor
Salix amygdaloides peach-leaf willow	Dioecious		May–June	July	Small, 2-valved capsule contains hairy seeds	14–18 seeds / capsule	
Salix bebbiana Bebb's willow	Dioecious		Apr–June	May–June	2-valved capsule; 24–48 capsules/catkin	5–7 seeds / capsule; 144–311 seeds per catkin	
Salix discolor pussy willow	Dioecious	Staminate catkins soft, silky	May		2-valved capsule	8–12 / capsule	
Salix exigua sandbar willow	Dioecious		May–July	May–June	2-valved capsule	15–36 / capsule	
Salix lucida ssp. *lasiandra* Pacific willow	Dioecious		Apr–May	June–Aug	2-valved capsule	12–20 / capsule	
Salix scouleriana Scouler's willow	Dioecious		Apr–June	May–July	2-valved capsule		

TABLE 3.2 *Seed production characteristics of conifers native to British Columbia (Eremko et al. 1989). (Cones refer to female cones only.)*

Species Common name	Cone length (cm)	Cone-bearing age (years)	Years between crops	Viable seeds per hectolitre of cones	Position of cones in crown	Ease of cone detachment
Abies amabilis amabilis fir	9–13	20	2–3	30 389	Top ¼	Difficult
Abies grandis grand fir	5–12	50	2–3	50 776	Top ¼	Difficult
Abies lasiocarpa subalpine fir	6–12	20	2–4	40 582	Top ¼	Difficult
Chamaecyparis nootkatensis yellow-cedar	0.5–1.5	Unknown	4 or more	93 965	Throughout	Easy
Larix laricina tamarack	1.5	40	3–6	32 000	Non-shaded part of crown	Moderate
Larix occidentalis western larch	2–3	25	1–10	119 312	Non-shaded part of crown	Moderate
Picea glauca white spruce	3–6	40	6	347 163	Top ⅓	Moderate
Picea mariana black spruce	2.5	10	4 or more	108 000	Top ¼	Difficult
Picea sitchensis Sitka spruce	5–10	25–40	3–4	194 270	Top ⅓	Moderate
Pinus albicaulis whitebark pine	3–8	20–30	3–5	515	Throughout	Difficult
Pinus contorta shore pine	3–6	15–20	2–4	coast: 176 660 interior: 70 546	Throughout	Difficult unless frozen
Pinus flexilis limber pine	7–20	20–30	2–4	6 454	Throughout	Moderate
Pinus monticola western white pine	10–25	20	3–7	7 687	Top ¼	Moderate
Pinus ponderosa ponderosa pine	7–9	12–16	4–6	31 522	Throughout	Difficult
Pseudotsuga menziesii Douglas-fir	5–10	20–25	2–10	coast: 39 577 interior: 70 343	Top ½	Easy
Thuja plicata western redcedar	1–2	20–30	2–4	897 837	Throughout	Easy
Tsuga heterophylla western hemlock	2–3	25–30	3–4	366 698	Throughout	Easy
Tsuga mertensiana mountain hemlock	2–8	30	3–6	356 428	Top ⅓	Easy

number of reproductive buds or, in other cases, can stimulate prodigious production of cones. Plant growth regulators (PGR) such as gibberellins have been used to increase cone production in conifer seed orchards (Ross and Bower 1989; Ross 1991), but PGR levels are difficult to alter, for logistical reasons, in natural stands.

Pollen, produced in male cones or anthers, is transported to female cones or flowers in the process of pollination. Successful pollination results in the fertilization of ovules; ovules then develop into seeds. Reduced pollination efficiency may be due to low pollen-cloud densities (few pollen-cone buds initiated), climatic conditions (e.g., rain, frost), or the presence or absence of pollen vectors. In conifers, which are wind pollinated, the absence of wind, or barriers to wind may inhibit pollination. Thus, the positioning of cones relative to tree height or relative to the windward and leeward sides of a tree can influence the frequency of filled seeds (Smith et al. 1988). In angiosperms, animals (insects, birds, and mammals) usually are the primary vectors of pollination. Pollen is sometimes dispersed by wind, but generally angiosperm pollination is less affected by climatic variables, although extreme conditions (cold temperatures, heavy rain) may still affect pollination success.

Fertilization efficiency may be reduced due to poor female cone production, self-pollination (which often results in embryo abortion), lack of pollen tube growth, or freezing temperatures (Shearer and Carlson 1993). Some conifers (e.g., Douglas-fir) can produce seeds (megagametophyte, but no embryo) without fertilization, but other conifers (e.g., pines) require the presence of pollen to form seeds (Owens and Molder 1984b). Additional background on the sexual reproduction of conifers may be found in Owens and Molder (1984a, 1984b, 1984c, 1984d, 1985).

Once fertilized, seeds may fail to mature due to abortion (which may be caused by self-incompatibility, insects, or disease), or because of climatic conditions during embryo development, particularly cool, cloudy weather during the summer (Eis 1976; Zasada et al. 1978). Some conifers do not shed their seeds when they mature in the fall, and instead may retain

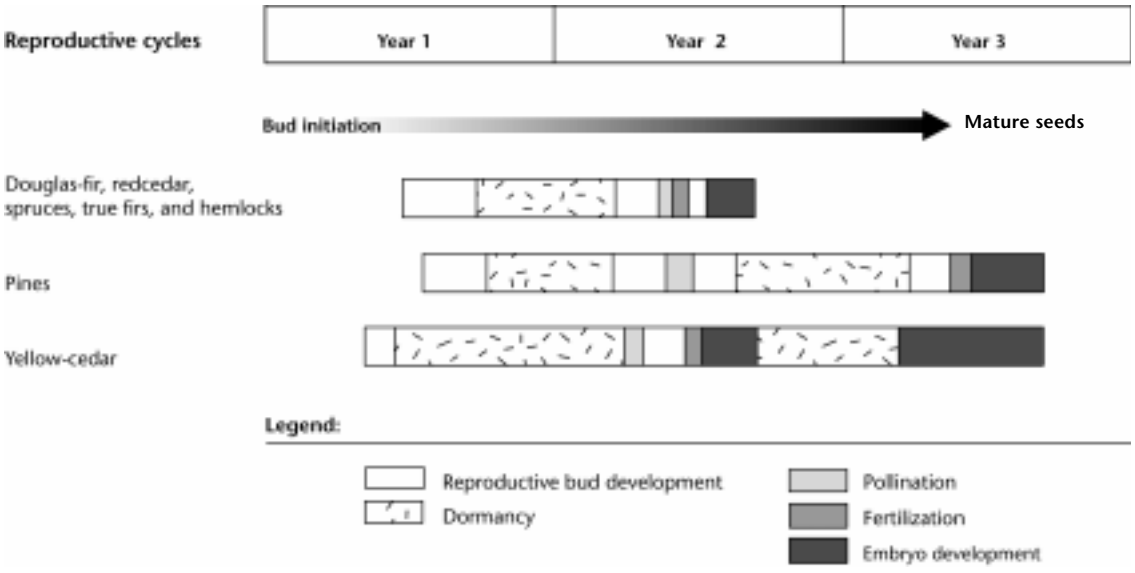


FIGURE 3.1 *Typical development and maturation cycles of British Columbia conifer seeds (Leadem 1996, adapted from Eremko et al. 1989). Most angiosperms exhibit a reproductive cycle similar to that shown for Douglas-fir, redcedar, true firs, and hemlocks.*

their seeds in the cones several years, a phenomenon referred to as serotiny (see Section 3.5.1).

Further information on tree seed biology may be found in Leadem (1996).

Seed production studies are usually undertaken to determine:

- the quantity and quality of seeds that may be produced relative to some variable, or
- the reasons for seed loss.

Other, more specific objectives may include:

- to predict the frequency of good seed crops (e.g., relative to climatic variables);
- to relate seed production to stand, tree, or crown characteristics;
- to examine the relationship between pollen abundance and filled seeds per cone;
- to relate the number of seeds per cone to cone age or cone size;
- to determine the relationship between the number of seeds in the cone half-face and the total number of filled seeds per cone;
- to determine the date of cone and seed maturation; or
- to establish the relation between seed quality and collection date, or cone handling methods.

Several examples of seed production studies are described as case studies in Section 3.8.

3.1.1 Collecting stand and study plot information

Before conducting seed production studies in natural stands, data should be collected on the tree and stand characteristics known to influence natural seed production. Examples are:

- density (number of seed trees per hectare);
- spatial arrangement of seed trees;
- age of seed trees;
- evidence of past production;
- evidence of animal use (e.g., squirrel caches, cones that have been broken or split);
- height and diameter at breast height (dbh);
- assessments of the general health and vigour of the crowns; and
- basal area values (in square metres per hectare). Specific selection criteria may be included, for example the basal area of all Engelmann spruce trees with dbh of 25.4 cm and larger, because trees of this size probably would be sufficiently mature to produce seeds (McCaughey and Schmidt 1987).

Once a site has been selected, data on individual trees may be collected. Examples of the data that could be included are (Alexander et al. 1986):

- dbh to the nearest 0.25 cm (trees 9.1 cm dbh and larger);
- total height, to the nearest 0.15 m;
- crown class;
- species;
- average length of live crown to the nearest 0.15 m (average of four sides); and
- average width of live crown to nearest 3.0 cm (average of two measurements).

3.1.2 Determining sample size

The choice of sample size, such as the number of seeds to sample per tree, can be made by applying statistical efficiency calculations to a preliminary set of measurements (Sokal and Rohlf 1981; Ager and Stettler 1983). See also Stauffer (1981, 1982) for sample size tables prepared specifically for forestry applications.

Sample sizes for measuring cone characteristics will depend on the species and the sites from which the cones were collected. Carlson and Theroux (1993) randomly selected 20 cones each from some subalpine larch, hybrid larch, and western larch collections. Only five cones were selected from six other western larch collections because initial sampling error estimates indicated that five cones would be adequate. Sample sizes for seed measurements should also be determined before the study. For a study of western larch and subalpine larch, length, width, and thickness were measured on only 10 seeds randomly selected from each lot; initial sampling estimates indicated this would enable standard errors to within 20% of the mean (95% confidence) (Carlson and Theroux 1993).

Environmental changes may result in year-to-year variations in cone and seed measurements. Ponderosa pine seeds collected in 1971, 1974, and 1976 showed negligible differences in seed weight, length, and width when comparisons were made within the same year. However, differences were found in all three measures when year-to-year variations were removed by adjusting values to be relative to those observed in 1971 (Ager and Stettler 1983).

3.2 Predicting Natural Seed Yields

It is often desirable to be able to predict the occurrence of natural seed production, to better understand what factors promote seed crops, to determine whether seed production will be great enough to merit collection of the crop, or to provide advance notification for organizing pre-collection activities. In the sense used in this section, a distinction is made between prediction and correlation. *Prediction* is the objective of these studies (we are trying to predict natural seed production) and *correlation* is the means to do so (correlations with various variables are used to predict the size of the crop).

3.2.1 Correlation with weather variables

Many models for predicting the size of natural seed crops have been developed, and those based on climatic variables indicate that the influence of weather conditions may be cumulative. In Douglas-fir and grand fir, a cool, cloudy summer 24–26 months before crop maturation appears to be a prerequisite for abundant lateral bud initiation. These conditions must be followed by cold, sunny weather through the winter (20–28 months before maturation); a wet April (16 months in advance) to promote lateral bud differentiation; and a warm, dry, sunny June before pollination (14 months before maturation) (Eis 1973a, 1973b). (See Figure 3.2 for a summary.) The importance of dry summers to floral initiation has also been demonstrated in other species, such as spruce, larch, and ponderosa pine (Eis and Craigdallie 1983).

For estimates of Douglas-fir, grand fir, and western white pine cone crops, Eis (1973a, 1976) counted cones on one side of mature trees in July. Counting was done using 10-power binoculars mounted on a tripod at a permanent station that offered a good view of the crown. Cone counts were multiplied by conversion factors (obtained by comparing binocular observations with physical cone counts on felled trees). Weather variables were derived from various expressions of temperature, precipitation, sunshine, and wind velocity. Starting 29 months (41 months for western white pine) before the cones matured, cone estimates were correlated with all monthly meteorological parameters. Where several meteorological variables were important in the same month, the data were combined and analyzed by stepwise, forward, multiple regressions.

Caron and Powell (1989b) correlated annual production of black spruce seed cones with warm weather in early May and early July and with low June rainfall, all in the year preceding maturation. Cone production data were recorded branch-by-branch during later spring. Seed-cone estimates of previous crops were obtained from a combination of (1) cones persisting on the trees, (2) stubs and basal cone scales left on the bearing shoots where squirrels had removed cones, and (3) cones or stubs on nearby shoots of comparable size and position within the crown when shoots of bearing type had been removed.

Mosseler (1992) used accumulated growing degree-days (GDD) to predict when cones of black spruce and white spruce could be collected without

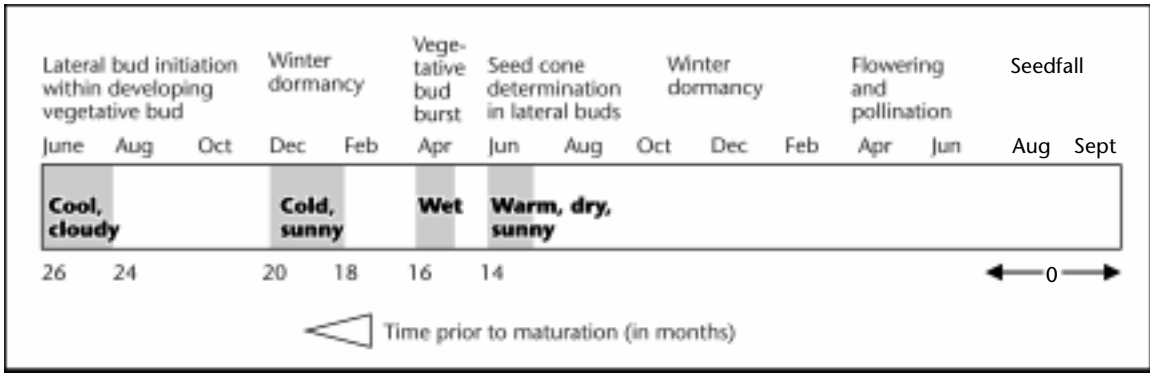


FIGURE 3.2 *Climatic conditions required for cone crop production in Douglas-fir (Eremko et al. 1989).*

adversely affecting seed quality. Accumulated GDD is a cumulative sum of the degrees of temperature above 5°C counted on each day that the daily mean temperature exceeds the 5°C threshold. In this study, Mosseler based the GDD on the simple mean of the maximum and minimum daily temperatures recorded at the Atmospheric Environment Service (Environment Canada) weather station nearest to each site. Cones were harvested at intervals of 100 GDD beginning at about 600 GDD. Mosseler found that natural seed release in white spruce occurred between 1200 and 1250 GDD. Cones from black spruce can be collected as early as 900 GDD and white spruce as early as 1100 GDD without significant losses in seed yield or quality. Similar results were found for white spruce in Alaska (J. Zasada, pers. comm., 1996).

Note that when attempting to correlate environmental factors to seed production, it is important to place sensors as near as possible to where pollen and seed cones are produced to ensure you are monitoring the conditions actually present in the canopy. Also, because comparable events in the reproductive cycle are not always synchronous, male and female flowers, for example, may not experience the same climatic conditions, so the environmental effects may be different. In paper birch, male flowers are induced in May before bud burst and thus must depend on resources stored in overwintering materials. Female flowers develop in late June to early July, so they are able to draw on current metabolites for their growth (Macdonald and Mothersill 1987).

3.2.2 Correlation with aspect and slope

Aspect and slope can significantly affect cone production, especially in northern regions. For example, black spruce trees growing on southerly aspects bore 2.5 and 5 times more seed cones and pollen cones, respectively, than trees growing on northerly aspects (Simpson and Powell 1981). Variations in slope and aspect can be difficult to depict, yet Simpson and Powell effectively conveyed their results by using concentric circles to show the percentage of cones produced in all compass directions (see Figure 3.3).

3.2.3 Correlation with crown size and crown class

In a closed canopy, the crowns of the trees forming the canopy are touching and intermingled so that light cannot directly reach the forest floor. However, dominant trees have crowns extending above the general level of the canopy and thus receive full light from above and partly from the side. The crowns of codominant trees, which form the general level of the canopy, receive full light from above, but comparatively little light from the sides. The relatively more favourable light environment for tree crowns in the upper canopy appears to enhance the cone production of dominant and codominant trees.

For example, dominant and codominant crown classes of Engelmann spruce produced three-quarters or more of the total seedfall in an experimental forest in the Colorado Rocky Mountains (Alexander et al. 1986) (see Case Study 1, Section 3.8). Also in black spruce, dominant trees produced almost three times

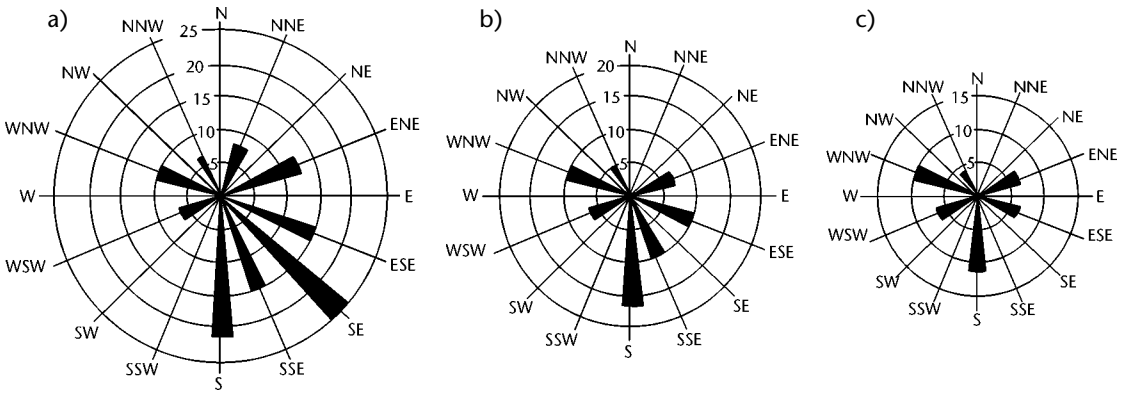


FIGURE 3.3 *Percentages of black spruce trees (concentric circles) 8–12 years old from seed, growing on slight (2–12%) slopes and on various aspects (Simpson and Powell 1981), which in 1980 bore: (a) more than five, (b) more than 15, and (c) more than 25 pollen cones.*

as many cones as codominant or the intermediate trees. Intermediate trees, on the other hand, produced about twice as many seeds per cone as dominant trees (Payandeh and Haavisto 1982) (see Case Study 4, Section 3.8). Note, however, in exceptionally good cone years, trees in all crown classes produce cones, not just the dominant and codominant trees (J. Zasada, pers. comm., 1996).

One possible reason for the periodicity observed in conifers is the substantial drain that cone production evidently places on the tree's resources. In Douglas-fir, decreased needle, shoot, and xylem ring growth was noted in good seed years in the trees that regularly produced cones (Tappeiner 1969). No such reductions were seen in trees that did not produce cones. Similar effects have been seen in grand fir, western white pine (Eis et al. 1965), subalpine fir, and mountain hemlock (Woodward et al. 1994).

Seki (1994) wanted to know the specific location of resources used to produce seeds in *Abies mariesii*, so he studied the allometric relationship between cone production and the productivity of the entire crown and of cone-producing branches. He concluded that total cone production was related to the square of the trunk diameter just below the lowest living branch (D_B^2 in Figure 3.4). He concluded that cone production was not related to the total amount of dry matter in the tree, but rather to the amount of dry matter accumulated in the neighbouring trunk and branches adjacent to cone production sites. Thus, cone production per branch depends on the resource status of a branch, to which at least part of the resources may be imported from other branches or the trunk for local cone production. In this way, the investment in seed production in individual branches may not necessarily cost the whole plant its vegetative growth or future survival.

3.2.4 Sampling methods using bud counts

Eis (1973b, 1976) developed a sequential sampling method to estimate white spruce and western white pine cone crop potential in the fall preceding the seed year. The method is based on the cumulative total count of female buds from one branch per tree collected from

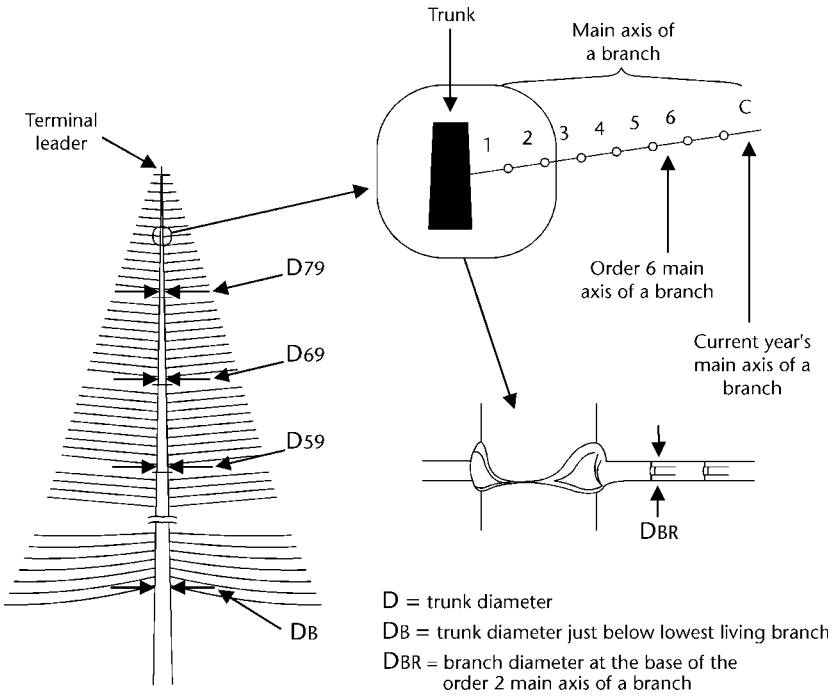


FIGURE 3.4 *Position of measurement for trunk diameters, the diameter of the base of a branch, and main axes for estimating of the number of cones (Seki 1994). Allometric relationships between various parts of a tree can be used for relating cone production to tree dimensions.*

the third whorl from the top. Bud counts from three terminal nodes on a branch of the fourth or fifth stem node may also be used, but with slightly lower accuracy. Trees should be 45–80 years old, 15–18 m high, of dominant class, with well-developed crowns. The observer must be able to distinguish reproductive buds (both male and female) from vegetative buds (identification based on general morphology, location in the crown and along the branch, and colour). When the cumulative bud count falls between given limits, cone crop potential can be classified with 80% probability, and no further samples are required.

Male pollen buds can be used in birch and alder to indicate the next year's seed production. Male buds are easily identifiable any time after September, and can be counted from the ground to provide reasonable estimates of female catkin production the following spring (J. Zasada, pers. comm., 1996).

A multistage variable probability sampling method, originally developed to estimate seed orchard efficiencies, could be applied to assess seed production in natural stands. Bartram and Miller (1988) first implemented a standard multistage approach in many seed orchards over several years. The effectiveness of this approach was evaluated against several alternative methods using the efficiency data initially collected for the study. Refer to the original paper for an example using this methodology in coastal Douglas-fir seed orchards in British Columbia. Mattson (1978) also suggested a multistage approach to evaluate red pine cone and seed production. In this scheme, the first stage is based on weather factors and the second stage on insect predators.

3.2.5 Scales for rating cone crops

Crop rating is an operational assessment procedure used by the B.C. Ministry of Forests to determine whether developing cone crops are collectable (Eremko et al. 1989). Suitable stands are located, and the relative size of developing cone crops is assessed in late June or early July. A visual assess-ment is made of the relative number of cones in the cone-bearing portion of dominant and co-dominant tree crowns. The number of cones on each cone-bearing tree and percentage of trees bearing cones in the stand are also assessed. Observations are grouped into classes depending on the relative number of cones observed on the tree (Table 3.3).

TABLE 3.3 *Cone crop rating based on the relative number of cones on the trees (Eremko et al. 1989) based on dominant and codominant trees only*

Class	Rating	Definition
1	none	No cones
2	very light	Few cones on less than 25% of the trees
3	light	Few cones on more than 25% of the trees
4	light	Many cones on less than 25% of the trees
5	medium [a]	Many cones on 25–50% of the trees
6	heavy [a]	Many cones on more than 50% of the trees
7	very heavy [a]	Many cones on almost all of the trees

[a] Crops rated as class 5 or higher are generally considered collectable.

The rating of potential cone crops is highly subjective and dependent on the surveyor's experience. The number of cones produced—and their distribution through the crown—varies considerably with tree species. Thousands of cones can constitute "many" on a spruce tree; the same number could be classed as "few" on a mature cedar.

A method based on seedfall data has been used in Oregon and California for rating cone crops of *Abies*, *Pseudotsuga*, *Tsuga*, and *Chamaecyparis* (Zobel 1979). Seeds were collected from traps approximately once a month over a 2-year period. The monthly trap samples from a site were usually combined, except where seed production was high enough to separately count individual traps. Basal area of each tree species over 15 cm dbh in each stand was measured using a wedge prism, with each trap as a sample point. Seed production effectiveness of a site was expressed as the annual seedfall per square metre of basal area of each species.

In another study, seed production of Engelmann spruce was based on seeds captured in traps and grouped into categories (Table 3.4).

Note that there may be some discrepancy between the cone crop rating and the number of seeds collected in seed traps. Such discrepancies can occur

TABLE 3.4 *Rating of seed crops by number of filled seeds per hectare (Alexander et al. 1982)*

Filled seeds per hectare	Seed crop rating
< 25 000	Failure
25 000–125 000	Poor
125 000–250 000	Fair
250 000–625 000	Good
625 000–1 250 000	Heavy
> 1 250 000	Bumper

in areas where there is heavy predation of cones by squirrels. Thus, generally it is best to count when cones have attained maximum physical dimensions, and before squirrels begin to harvest.

Cone crop estimates also can be obtained by direct sampling of cone-bearing regions or fertilized flowers. For example, cone crops of eastern redcedar (*Juniperus virginiana*) were estimated by multiplying the average number of cones per sample branch (5–10) in October by the cone-bearing canopy foliage (Holthuijzen et al. 1987).

3.2.6 Monitoring the seed crop

The sequence and length of different components of the reproductive cycle must be understood when planning and executing seed production studies because different components are not the same in each species. For example, you need to know the length of the entire reproductive cycle of each species involved in the study, since this will determine when monitoring should begin. You must also know the timing of other critical events, such as pollination, fertilization, and periods of bud dormancy, to ensure you are at the right place at the right time.

For Douglas-fir, redcedar, spruces, true firs, and hemlocks, the development and maturation cycle takes about 16 months (Figure 3.1). For these species, male and female strobili appear in the spring, and seeds mature in the fall of the same year. In maple, alder, birch, Garry oak, and willows, seeds are also produced in the same year as the female flowers. However, in pines, complete development takes 26 months because fertilization is delayed for 1 year after pollination. In yellow-cedar, pollination and fertilization take place in the same growing season, but the total cycle usually lasts about 28 months. Under natural conditions, seed maturation is delayed by a period of dormancy until the following year (Figure 3.1); however, under favourable conditions in seed orchards, pollination, fertilization, and seed maturation can occur within the same year (El-Kassaby et al. 1991).

In conifers, male and female strobili appear on the same tree (with the exception of yew). However, the distribution of cones within the crown varies with the species (Table 3.2), and even within the same species, male and female cones may occur in different parts of the crown. In hardwoods, it may be necessary to identify male and female clones before monitoring, since dioecious hardwoods, such as *Salix*, *Populus*, and *Fraxinus*, bear male and female flowers on different trees.

Monitoring pollen

Pollen abundance in the air during female receptivity is believed to be closely related to seed production. Estimates of pollen abundance can be used to formulate a relationship to (1) total seed production for the stand, and (2) total filled seeds per cone. In lodgepole pine, the number of male meristems produced on individual trees was correlated to the frequency of filled seeds on those trees (Smith et al. 1988). Weather data for previous and current year also can be incorporated to develop predictive models for seed yield. Possible relationships that can be studied are the amount of rain during flower initiation and temperature minima during critical stages of cone maturation (Stoehr and Painter 1995).

Many different kinds of pollen samplers have been used to assess pollen abundance. Each has its advantages and disadvantages, but often the choice depends on the financial resources available. The least expensive approach is a microscope slide placed on a flat surface or on the ground. The results from such a method may have little relationship to the actual densities experienced at the female cone level, but it may be possible to correlate the data with crown measurements.

Another inexpensive method used in Sweden and Finland is to trap old pollen strobili that fall to the ground to obtain estimates of pollen cone production (Leikola et al. 1982). At the other end of the spectrum

are automated air samplers that pull a known volume of air through the sampler so that air movement is dynamic. This differs from strip chart recorders, which are "passive" and depend on natural wind and air movement to adhere pollen to the "sticky" surface.

The abundance of pollen in conifer stands can be estimated using a 7-day pollen monitor (Webber and Painter 1994). Several monitors (three is good, depending upon the size of the plot) should be placed in the experimental sites 1 week before expected pollen shed and left in the field until the pollination period is completed. The monitor is mounted on a pole 4 m above the ground and always turns into the wind. The monitor consists of a permanent chart wrapped around a drum, which is rotated with a clock mechanism. The chart is made of mylar coated with petroleum jelly so that pollen will adhere; heating the petroleum jelly slightly creates a smooth, even coat. Since the drum completes one turn each week, pollen charts must be changed weekly. Pollen charts are assessed in the laboratory, where pollen densities, timing of dispersal, and pollen identification can be determined on a daily basis. Proper evaluation of pollen charts depends on the experience and expertise of an analyst familiar with pollen density patterns.

Sarvas (1962, 1968) used different types of pollen samplers to estimate pollen density in Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*), and was able to closely relate flowering to heat-sum calculations. He placed all his pollen samplers on towers at the height of the female flowers. Zasada et al. (1978) used this method for white spruce. Sarvas also used a small globe sampler to measure pollen rain density. The globe shape was chosen because it more closely resembled the shape of a female cone, and thus better approximated the pollen density patterns expected on a "cone-shaped" surface.

Another pollen trap described by Caron and Powell (1989a) consists of a glass rod (7 mm diameter × 10 cm long); one end is coated with a thin layer of white petroleum jelly to serve as the catching surface, the other end is tightly fitted through a rubber stopper into a hole on a wooden base. The square wooden base is grooved to slide into a holder, which can be set so that the edges are aligned to the four cardinal directions. Each trap holder is protected from rain by polyethylene film installed on a wire frame 15–20 cm above the base. A vial (25 mm diameter × 95 mm long) with a fitted rubber stopper is used to protect the catching surface from contamination before installation and after collection. The traps are collected and replaced daily.

Smith et al. (1988) sampled airborne pollen for density estimates of lodgepole pine at canopy level near the centres of two squares that made up a central rectangle surrounded by a 10 m bank. Air samples were taken at 20-minute intervals over 17 or 21 days with Kramer-Collins air samplers (Kramer et al. 1976). Pollen counts averaged over the 7 peak days were used to calculate relative pollen densities in the 127 stands chosen for study. Pollen cone production was estimated by counting the number of terminal meristems producing male strobili. Using binoculars, terminal meristems were counted for either the entire tree, or 100 or 200 meristems were counted on a portion of larger trees, and that portion was estimated as a percentage of the total surface area to provide a meristem total for the tree. Estimates ranged from 0 to 8000 male meristems per tree. To check the consistency of the technique, 131 trees were counted on consecutive days. Of the 114 counts that differed on the 2 days, the second day had the smaller count 52 times. The smaller count averaged 75% of the larger count for the 126 trees that had male meristems. Using a similar sight estimation of female cones on trees that were later cut down so that cones could be counted, Elliott (1974) and Smith (1981) found that their under- or overestimation deviated from the actual count by an average of 21%.

Pollen from different species can usually be identified microscopically (Figure 3.5). In a study of black spruce, the pollen catch was systematically examined (100-power magnification) on the four directional faces of each trap (Caron and Powell 1989a). Pollen identification to the species level (or at least genus) was accomplished by comparing pollen samples collected directly from trees with micrographs and species descriptions (Richard 1970; Adams and Morton 1972). In mixed stands comprised of species with similar-looking pollen, it is advisable to install additional pollen monitors in pure stands of the species located near to the study site. Pollen density records from mixed stands can then be compared to those from pure stands to determine the relative pollen abundance and time of pollination of different species (Stoehr and Painter 1995).

Monitoring seed cones

This section focuses on monitoring female conifer cones, although the same procedures could also be used for the flowers, fruits, and seeds of hardwood species.

To sample for cone production (either male or female cones) it is necessary to determine where the cones are produced. In western larch, most seed cones are produced on ascending branches or on recent terminal leaders within the upper third of the crown; most pollen cones are found on horizontal or descending branches within the lower two-thirds of the crown (Shearer and Schmidt 1987). In British Columbia considerable overlap of the seed and pollen cones occurs within the lower half of the crown (Owens and Molder 1979a, 1979b) (Table 3.2).

In species such as whitebark pine, cone scars can be monitored to estimate past cone crops (Morgan and Bunting 1992) (Figure 3.6). Morgan and Bunting chose a 90 m transect that contained 10 mature, cone-producing whitebark pine trees with crowns

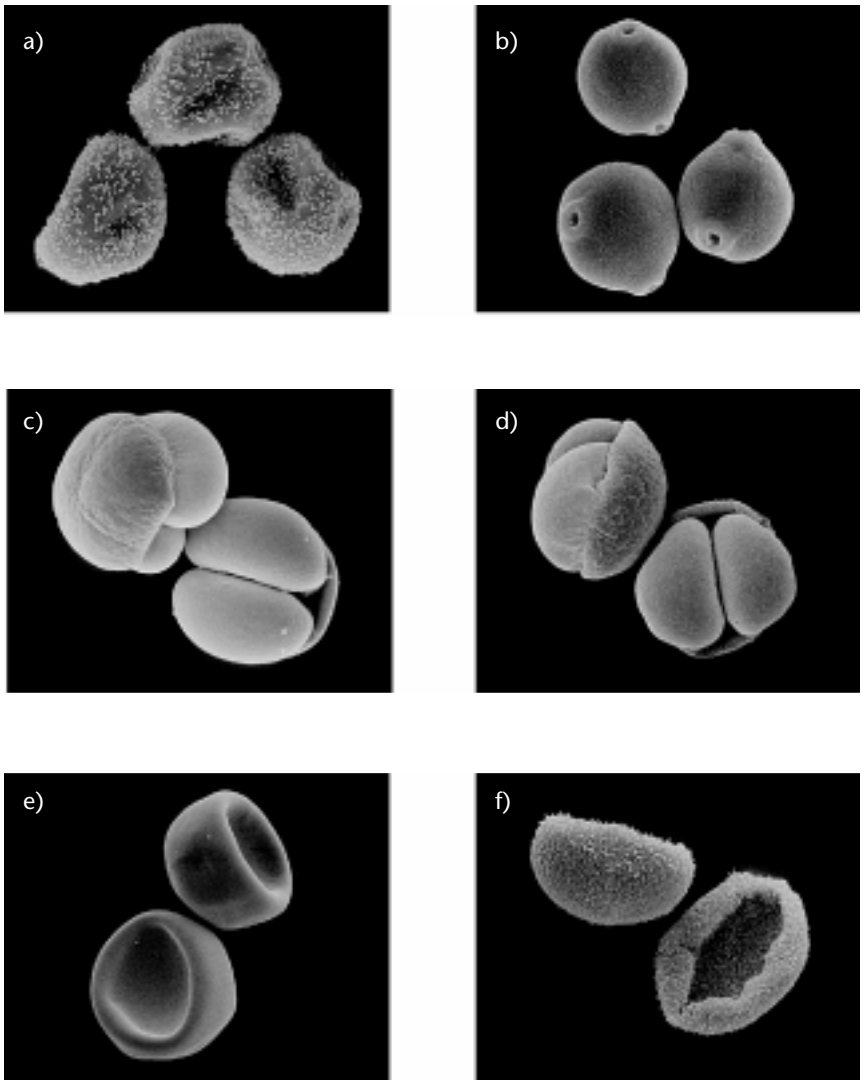


FIGURE 3.5 *Scanning electron micrographs showing whole pollen and details of the exine. (a),* Chamaecyparis nootkatensis *(×1100); (b),* Betula *(×860); (c),* Abies amabilis *(×400); (d),* Pinus contorta *(×720); (e),* Pseudotsuga menziesii *(×360); (f),* Tsuga heterophylla *(×540). (Owens and Simpson 1986).*

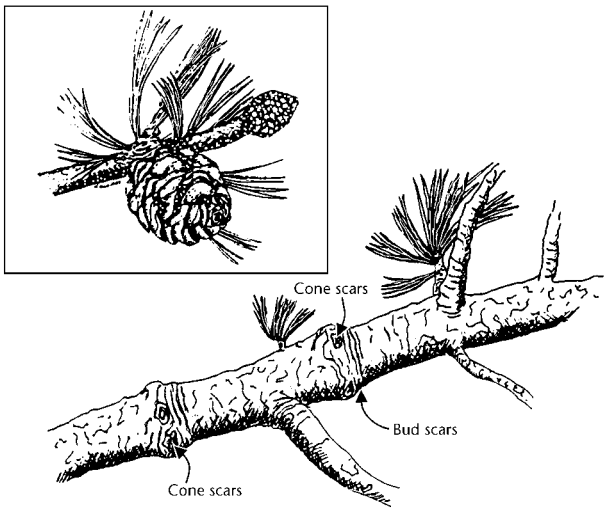


FIGURE 3.6 *The oval, raised cone scars of* Pinus albicaulis *can be counted and aged by the nearby annual bud scars on twigs (Morgan and Bunting 1992).*

readily visible from the ground from at least two angles. The data were obtained by visiting each transect in late July or early August (before appreciable harvesting by red squirrels and Clark's nutcrackers); binoculars were used to count the mature cones visible from the ground. Each tree was climbed to access cone-bearing branches normally found in the uppermost portion of the canopy; cone-bearing branches are visibly shorter and stouter than other branches. Five branches were sampled that bore either cones or recent cone scars. The mean number of cones or cone scars was calculated for each sampled stand. The transformed values of the nonzero data were standardized by calculating *z* (the difference between the individual observation and the means of all observations, divided by the standard deviation of all observations). The *z*-scores were calculated separately for scar and cone counts, then combined for classification into poor, average, or excellent cone crops. Morgan and Bunting recommend counting immature cones as an index of cone production in the following year. Another method involves counting immature cones on high-resolution aerial photographs. The immature cones are readily visible from above; they occur at the ends of upswept branches near the top of crowns and their deep purple colour contrasts with the foliage.

Similarly, in Douglas-fir, it is possible to trace pedicle remains to estimate previous cone production (Tappeiner 1969). In *Abies*, cone spindles remain on branches for several years after the cones have disintegrated; these also might be used to estimate previous production (J.C. Tappeiner, pers. comm., 1997).

In a study of ponderosa pine, four branches in the upper half of the crown were randomly selected and permanently marked for the presence of male and female flowers (strobili), conelets, and mature cones (Heidmann 1984). Flowers were counted in July of each year for 4 years. Because of the great number of male flowers on some branches (as many as 150 clusters per branch), an average was determined for a sample of 20 clusters, then multiplied by the number of clusters to obtain the total flower count for that branch. All female flowers were counted.

Fourteen western larch stands ranging in age from 46 to 100 years were monitored by Shearer and Carlson (1993) in Idaho, Montana, Oregon, and Washington. Using binoculars, they estimated the number of new seed cones in spring. Five trees with the greatest seed cone counts were climbed and the number of developing cones was estimated by counting the number of branches with seed cones, and new seed cones (living and dead) on six random branches (two from each third of the crown). The number of potential seed cones was estimated by multiplying the average number of cones per sample branch by the number of cone-bearing branches. Seed cone survival was estimated in August by counting the number of cones that matured on the six branches selected in the spring. Seed cone mortality was determined by subtracting surviving cones from the total cones counted at the first visit of the year. During the first visit, researchers marked 25 cones on the two trees bearing the most cones at each site. During subsequent visits they documented cone development, as well as the time and cause of cone damage. Dead cones were removed and the probable cause of death was identified.

Cone and seed analysis

Initially developed for southern pines, cone and seed analysis is an excellent procedure for identifying actual and potential seed production and the causes of seed loss in conifers. Bramlett et al. (1977) provide complete background and procedures.

Cone and seed analysis is based on calculating four critical ratios (efficiencies), which are used to identify the sources (stages) in which major losses occur:

$$\text{cone efficiency} = \frac{\text{number of cones harvested}}{\text{number of conelets initiated,}}$$

$$\text{seed efficiency} = \frac{\text{number of filled seeds}}{\text{number of fertile sites,}}$$

$$\text{extraction efficiency} = \frac{\text{extracted seeds per cone}}{\text{total filled seeds per cone, and}}$$

$$\text{germination efficiency} = \frac{\text{number of germinated seeds}}{\text{total filled seeds.}}$$

The analysis requires the determination of:
- the potential number of seeds per cone;
- the total number of seeds per cone;
- the number of extracted seeds per cone;
- the number of filled seeds per cone; and
- the number of empty and insect-damaged seeds per cone.

Note that the number of filled seeds must be determined in addition to the total number of seeds. This is essential to reflect the actual seed production potential of the species.

Commercial services are available if you do not wish to perform your own cone and seed analyses (refer to Portlock [compiler] 1996).

Cone and seed analysis has been applied in British Columbia to analyze seed production of lodgepole pine and Douglas-fir (McAuley 1989a, 1989b). For the analysis, samples were randomly selected from five sacks among those filled that day. Random subsamples consisting of one cone per sack were placed in separate bags for subsequent analysis. Based on previous experience, a sample size of 40 Douglas-fir cones per orchard (45 cones for lodgepole pine) was considered to yield a reasonably precise estimate of single, orchard-level means. Samples for cone and seed analysis of western larch consisted of 20 cones collected from each of 20 individual trees per hectare (Stoehr and Painter 1995).

Hardwood trees could also be assessed using coneand seed analysis methods. Determining the cone and seed efficiencies would require some modifications since, in species such as *Salix* and *Populus*, catkins are comprised of capsules, each of which has several to many seeds. In *Alnus* and *Betula*, catkins are more like conifer strobili. In *Acer* and *Fraxinus*, fruits are paired or single samaras, respectively, each containing one seed per samara. Refer to Table 3.1 and to Section 3.3.1 for further information on hardwood fruit characteristics.

Whether the method is used for conifers or hardwoods, two cautions should be considered in conducting cone and seed analysis and extending the results to the species:

1. If at all possible, the cone analysis should be repeated during another good seed crop year.
2. If an unharvested stand can be found near the study plot, cones should also be collected from the unharvested stand for comparison.

Filled seeds per cone

Measurements of the number of filled seeds per cone are obtained primarily for predictive purposes. Numbers are used to plan the size of cone collections in a particular area, or to estimate the potential of a site for natural regeneration. Large samples are generally not needed. For example, in ponderosa pine only 20 closed cones from each lot were required to obtain good corre-lations between filled seeds per cone and kilograms of seeds per hectolitre of cones (Ready 1986).

Determining the total number of filled seeds per cone is time consuming, as it requires complete dissection of the cone. Special tools are needed as most cones are hard and woody. For these reasons many studies have attempted to relate the number of filled, sound seeds seen in the cone half-face to the total number of seeds in the cone (see Figure 3.7). Schmid et al. (1985) tested several sampling designs to determine the accuracy and precision of each design in estimating the mean numbers of filled seeds. They found that half-face counts on 20 cones (two cones from each of 10 trees) from a ponderosa pine stand estimated the filled-seed percentage for whole cones within ±10 units of the mean. Olsen and Silen (1975) multiplied the number of filled Douglas-fir seeds seen in the cone half-face by 4.5 for an estimate of the total seeds per cone. From each 7.6 L of undried cones, they cut 10 cones in half longitudinally,

counted the number of full seeds on one cut surface, then dried and extracted the cones to determine the total number of filled seeds.

Half-cone counts have been used extensively in British Columbia to determine whether developing cone crops are collectable. Recommended collection standards based on filled seeds in the cone half-face are given in Eremko et al. (1989) for *Abies amabilis*, *Abies grandis*, *Abies lasiocarpa*, *Chamaecyparis nootkatensis*, *Larix occidentalis*, *Picea glauca*, *Picea mariana*, *Picea sitchensis*, *Pinus contorta*, *Pinus monticola*, *Pinus ponderosa*, *Pseudotsuga menziesii*, *Thuja plicata*, and *Tsuga heterophylla*.

3.3 Determining Fruit and Seed Maturity and Quality

Understanding fruit and seed morphology is vital in designing and implementing a seed production study. A brief description of the seed-bearing structures of British Columbia tree species follows (summarized in Table 3.5). In addition, various cone and seed attributes (such as colour, weight, and length) can be used to indicate seed maturity. Assessment of seed maturity may be the objective of the study or may be important for obtaining the best-quality seeds for another study.

Because conifer and hardwood seeds can vary so greatly, the procedures for collecting, processing, and storing seeds of various species are discussed separately in Section 3.4 according to the characteristics of their fruits.

3.3.1 Description of conifer and hardwood fruits

The dry multiple fruit of a conifer is called a cone or strobilus (plural strobili). A female cone consists of a central axis supporting overlapping bracts, each of which subtends a scale bearing naked seeds. Gymnosperm, another term for conifer, means "naked fruit," referring to the fact that conifer seeds are borne

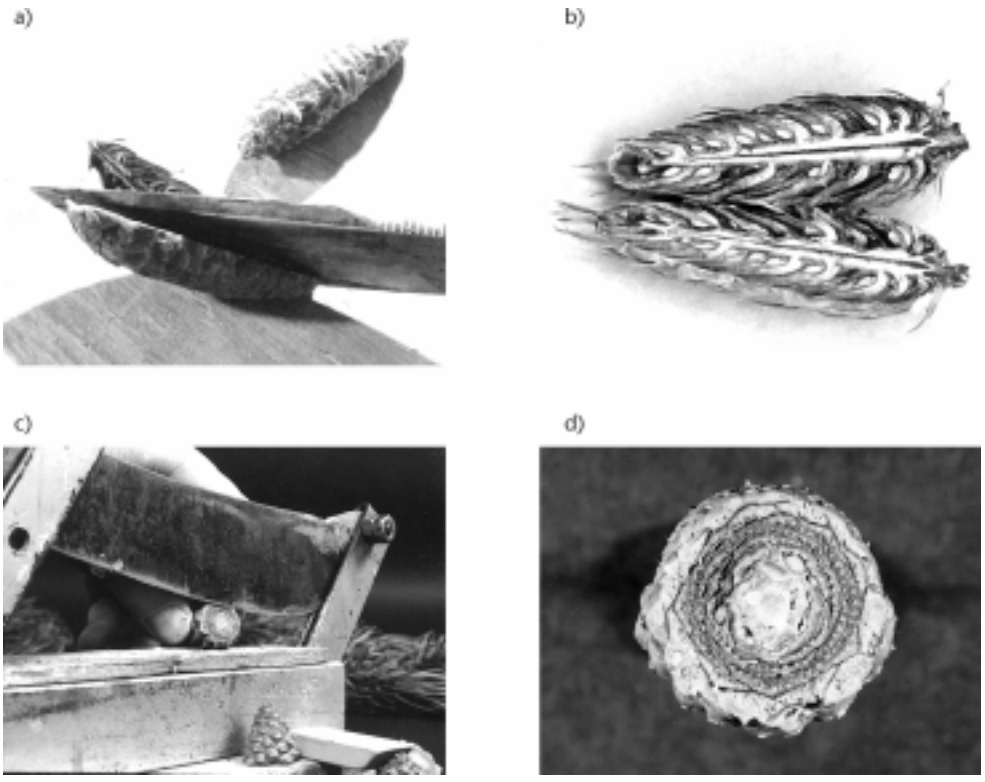


FIGURE 3.7 *Longitudinal and transverse sectioning of cones: (a) longitudinal sectioning of an interior spruce cone; (b) sectioned Douglas-fir cone; (c) transverse sectioning of a lodgepole pine cone; (d) sectioned lodgepole pine cone (Eremko et al. 1989). The number of filled, sound seeds seen in the cone half-face can be used to estimate the total number of seeds in the cone.*

naked on the ovuliferous scales of their cones. A male cone consists of a central axis supporting spirally arranged microsporophylls bearing pollen sacs that contain the pollen grains. Conifers in British Columbia produce several to numerous seeds in a single cone. An exception is Pacific yew, whose fruit is a red, berry-like aril that contains a single "naked" seed.

The seeds of hardwoods are enclosed in protective fruits that vary considerably in size, colour, and structure. The seeds of bigleaf maple and Oregon ash are contained in dry winged fruits called samaras. In maple, two winged seeds are joined to form a V, but in ash, each fruit contains only a single winged seed. The fruits of red alder, birch, poplar, and willow are

TABLE 3.5 *Seed-bearing structures of trees occurring in British Columbia*

Fruit type	Definition	Example
achene	Dry, indehiscent one-seeded fruit.	*Betula*
acorn	One-seeded fruit of oaks; consists of a cup-like base and the nut.	*Quercus garryana*
aril	Exterior covering or appendage that develops after fertilization as an outgrowth from the point of attachment of the ovule.	*Taxus brevifolia*
berry	Pulpy fruit developed from a single pistil and containing one or more immersed seeds, but no true stone.	*Arbutus menziesii*
capsule	Dry, many-seeded fruit composed of two or more fused carpels that split at maturity to release their seeds.	*Populus*, *Salix*
catkin	Spike-like inflorescence, usually pendulous, of unisexual flowers (either staminate or pistillate). Also used to describe the fruit. Compare *strobile*.	*Alnus*, *Betula*, *Populus*, *Salix*
cone	Dry multiple fruit of conifers. A female cone consists of a central axis supporting overlapping bracts, each of which subtends a scale bearing naked seeds. A male cone consists of a central axis supporting spirally arranged microsporophylls bearing pollen sacs that contain the pollen grains. Syn. *strobilus*.	all B.C. conifers, except *Taxus*
drupe	Fleshy indehiscent fruit, usually one-seeded, containing a seed enclosed in a hard, bony endocarp (pericarp). Syn. *stone fruit*.	*Cornus*, *Prunus*
nut	Dry, indehiscent, one-seeded fruit with a hard wall.	*Quercus garryana*
pome	Many-seeded fruit of the apple family consisting of an enlarged fleshy receptacle surrounding the papery, fleshy pericarp.	*Malus fusca*
samara	Dry, indehiscent winged fruit; may be one- or two-seeded.	*Acer* (two-seeded), *Fraxinus* (one-seeded)
strobile (pl. strobiles)	Spiky pistillate inflorescence or the resulting fruit; not a true strobilus. Syn. *female catkin*.	*Alnus*, *Betula*, *Populus*, *Salix*
strobilus (pl. strobili)	Male or female fruiting body of the gymnosperms.	all conifers, except *Taxus*

Notes:

carpel: simple pistil or single member of a compound pistil.

imperfect flower: flower which contains either, but not both, functional male or female parts.

indehiscent: refers to dry fruits that normally do not split open at maturity.

nutlet: nut-like fruit or seed, as in *Alnus* or *Betula*.

perfect flower: flower that contains both pistil and stamens.

pericarp: wall of a ripened ovary that is homogeneous in some genera and in others is comprised of three distinct layers: exocarp, mesocarp, and endocarp. Syn. *fruit wall*.

pistil (or pistillate): the female part of angiosperm flowers, containing the ovary, from which seeds develop.

staminate: referring to male angiosperm flowers, containing the stamens, from which pollen is produced.

stone: part of a drupe consisting of a seed enclosed in a hard, bony endocarp as in *Prunus* and *Cornus*.

catkins (or strobiles), which develop from the spike-like female flowers. The drooping catkins of poplar and willow comprise many capsules that split open at maturity to release many seeds per capsule. The catkins of birch break up at maturity to release the small winged nutlets. The female catkins of alder are woody cones; the cones contain oval nutlets that do not break up at maturity. The fruit of Garry oak is an acorn, consisting of a hard-coated nut in cup-like base.

Several hardwoods have fleshy fruits surrounding their seeds. The fleshy fruit of Pacific dogwood, bitter cherry, and cascara is a drupe, sometimes called a stone fruit. A drupe usually contains a single seed enclosed in a hard, bony ovary wall (the stone). The arbutus fruit is a berry, a pulpy fruit developed from a single pistil (female part of a flower) and containing one or more immersed seeds, but no true stone. The Pacific crab apple is a pome, a many-seeded fruit consisting of an enlarged fleshy receptacle surrounding a papery ovary wall.

3.3.2 Assessing embryo development

The most commonly used indicators of maturity in conifer seeds are cone and seed colour, degree of cone opening, condition of the megagametophyte, and length of the embryo (Edwards 1980; Shearer 1985; Eremko et al. 1989). Cones lose moisture as they mature, and cone colour usually changes from green to brown. In the field, specific gravity of the cones has been used to monitor maturation of Douglas-fir cones (Shearer 1985). The rate of maturation is influenced by the number of degree-days (Mosseler 1992), elevation (Shearer 1985), and latitude.

Conifer seeds should not be collected until embryos fill at least 90% of the embryo cavity (Figure 3.8). Although collection can begin when embryos fill 75% of the cavity, collecting seeds when embryos are more mature will result in better-quality seeds (Edwards 1980; Zasada 1988; Eremko et al. 1989).

Embryo development and size may be determined destructively or non-destructively. Non-destructive methods depend on an external visual assessment of the seeds. For example, with paper birch it is possible to distinguish viable well-filled seeds from non-germinable seeds by viewing the seeds with a dissecting microscope equipped with substage illumination (Bevington 1986). The small size of eastern

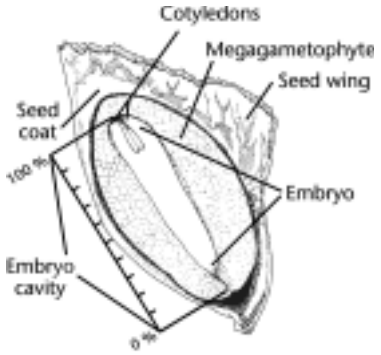


FIGURE 3.8 *Anatomy of a mature Douglas-fir seed. Ninety percent elongation of the embryo is the recommended standard for collection (Leadem 1984).*

white cedar (*Thuja occidentalis*) seeds makes it difficult to determine if the seeds are filled. Briand et al. (1992) therefore used swelling of the embryo area as a means of classifying developed from undeveloped seeds. Seed colour can also be used as a key to viability, and is discussed in more detail in Section 3.3.3.

Destructive methods of seed assessment include cutting seeds open to expose the embryo. This procedure allows for a greater variety of measurements, such as embryo length, embryo cavity length, and cotyledon length. Alternatively, you can germinate the seeds and determine the anatomical characteristics of the embryos. Cotyledon numbers of ponderosa pine were determined by germinating 20 seeds, and selecting 10 germinants for scoring (Ager and Stettler 1983).

See Sections 7.2.5 and 7.2.6 for quick tests and other viability tests.

3.3.3 Assessing seed colour

Colour is frequently used as an indicator of both cone and seed maturity. In some instances, the purpose of the study may be to assess seed colour as an indicator of seed maturity. In other instances, determining maturity (using colour) may be simply a tool to ensure that the best quality seeds are collected.

Seed colour is one of the more difficult indicators to quantify, as it relies on the subjective judgement of the observer, and cone and fruit colour can vary among different individuals of the population. Several instruments, such as Tristimulus colorimeters and video imaging systems (McGuire 1992), can be

used to quantify seed colour and reduce the subjectivity of colour readings.

Seed colour variation in ponderosa pine was quantified by constructing a 12-seed gradient with seeds from the entire collection (one seed from each of 12 trees) (Ager and Stettler 1983). Trees were then scored by comparing the adaxial (exposed) surface of five typical seeds from a given tree with the gradient. Mottled seeds were evaluated on overall shade. Although there were large colour variations among the populations studied, the seeds of a single tree were remarkably uniform when compared to seeds from different trees. This uniformity is probably a result of the high heritability and maternal control of seed morphology in pines (Kraus 1967).

Seed colour can be used as a key to the viability of willow and poplar. In willow the presence or absence of the embryo can be determined by the dark green of the cotyledons showing through the transparent seed coat.

3.3.4 Measuring cone and seed dimensions

Cone dimensions

Cone length can be measured using vernier calipers (0.05 mm precision) (Caron and Powell 1989a). Depending on the type of data presentation or data analysis, it may be convenient to group measurements of cone length into classes. Bergsten (1985) initially grouped cone length measurements of Scots pine into 18 classes (2.5 mm each) from 15.0 to 60.0 mm, but subsequently combined them into six length classes.

Temperature and humidity may affect some cone measurements. In western larch and subalpine larch, Carlson and Theroux (1993) measured cone length and diameter on both wet and dry cones, because moisture differentially influences their shape. They hydrated dry cones by placing them in a chamber at 100% humidity for 24 hours, then measured cone diameter at the midpoint along the longitudinal axis of the cone.

Cone measurements are sometimes used as stable taxonomic markers to distinguish between species of the same genera, and their hybrids. Carlson and Theroux (1993) measured the length and width of five scales and five bracts of western larch and subalpine larch, randomly selected from the middle one-third of each cone (measured when dry) to the nearest 0.01 mm. Bract length was measured from the base to the tip of the pointed apex; width was measured at the widest point of the bract.

Large differences in cone morphology may be noted between stands and between years. Abnormal cone morphology may also be observed, for example, "forked" cones, proliferated cones (with needles formed at the apex), and combinations of male and female in the same cone. (See Zasada et al. 1978 for examples in white spruce.)

Seed dimensions

Measurements of seed size (length, width, and thickness) will depend on the anatomical characteristics of the seed (Figure 3.9). In ponderosa pine, Ager and Stettler (1983) defined seed length as the distance between the micropylar and basal ends, and width as the maximum distance across the seed perpendicular to the long axis. Length and width data were based on five seeds per tree.

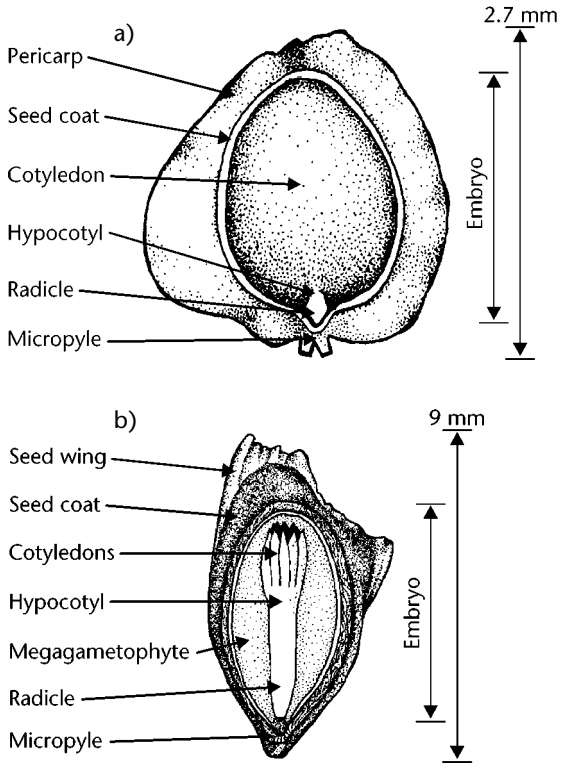


FIGURE 3.9 *Tree seed anatomy (longitudinal sections): (a) red alder, an angiosperm; and (b) Douglas-fir, a gymnosperm (Leadem 1996).*

In western larch and subalpine larch, Carlson and Theroux (1993) measured seed length, width, and thickness to the nearest 0.01 mm. Width and thickness were measured at the widest part of the seed, then each seed was sliced longitudinally. The thickness of the seed coat was measured to the nearest 0.01 mm midway between the base and apex of the seed. Sampling was done on 10 seeds randomly selected from each lot, as initial sampling estimates indicated that this sample size would enable standard errors within 20% of the mean with 95% confidence.

Briand et al. (1992) used a dissecting microscope equipped with an ocular micrometer to measure the small seeds of eastern white cedar (*Thuja occidentalis*). Seeds were positioned such that the micropylar end was facing up and the concave face of the seed was towards the viewer. The following measurements were determined to the nearest 0.1 mm: length and width of the seed and the embryo area, length of the right wing, and right wing width measured at the midpoint (Figure 3.10).

Extremely small seeds of *Salix* and *Populus*, which can be especially difficult (and tedious) to measure, can be graded by sifting them through a set of soil screens. Although this method is not as precise as using a micrometer, it is effective and less expensive (J. Zasada, pers. comm., 1996).

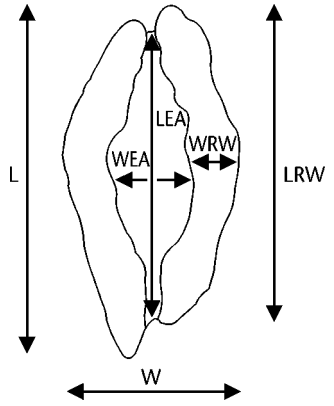


FIGURE 3.10 *Outline drawing of a typical seed of* Thuja occidentalis *(Briand et al. 1992) showing significant seed dimensions. LEA = length of embryo axis; W = width of entire seed; LRW = length right wing; WRW = width right wing; WEA = width embryo axis.*

If X-ray equipment is available, seeds can be placed on celluloid film and exposed to X-rays (see Section 7.2.6). Once developed, the films can be placed on a microfiche viewer (of the type commonly used in libraries). Precise seed dimensions can be obtained by direct measurement of the projected images. Actual and projected dimensions can be compared to calculate an appropriate conversion factor. If the size and shape of seeds are suitable, an overhead light projector and 35 mm camera film can be used in a similar manner.

Anatomical measurements were made on white spruce seeds by cutting the seeds longitudinally and measuring the embryo length, embryo cavity length, and cotyledon length with a micrometer mounted in the eyepiece of a binocular microscope (Zasada 1988). When multiple embryos were present, embryo measurements were made on the dominant embryo. Samples consisted of 10 white spruce seeds taken from the central portion of four cones from each tree. In many conifers, seeds at the apical and basal portions of the cone are poorly developed (Bramlett et al. 1977).

3.3.5 Estimating seed weight and volume

Seed weight can be expressed as the fresh weight (FW) or dry weight (DW) of seeds. The expression used for seed weight will depend on the context in which it is used. International seed testing rules prefer the use of fresh seed weight (before drying in an oven), whereas ecologists more often use the dry weight of seeds. See Section 7.2.2 where fresh weight and dry weight are more thoroughly discussed.

Seed weights should be determined to at least two significant figures. The sample size required to estimate seed weight varies with the species and the variability of the crop. For example, two 10-seed replicates per tree were used by Ager and Stettler (1983) to determine the weight of ponderosa pine seeds. International standards for sample sizes for weight measurements may be found in International Seed Testing Association (1993) or the Association of Official Seed Analysts (1993). Seed weights of tree species occurring in British Columbia are listed in Table 3.6.

For serotinous cones of species such as jack pine and lodgepole pine, the volume of cones can be determined by immersing individual cones in a graduated cylinder containing water and a wetting agent (Rudolph et

al. 1986). A similar procedure has been used effectively for white spruce cones (Zasada et al. 1978).

3.4 Collecting and Processing Seeds

In many studies, seeds are an end product by which successful reproduction is assessed. Thus, efficient methods of collecting, extracting, and storing seeds must be known. The method selected for collecting and extracting seeds depends on the species being studied. Conifers generally require some effort to extract the seeds from the cone, and hardwood seeds are enclosed in a hard or fleshy fruit which must be removed to obtain the seeds.

Another factor affecting seed collection is the capacity of seeds for long-term storage. All conifer seeds and many hardwood seeds can retain viability for long periods if seed moisture content (mc) is reduced to low levels (5–10%) and the seeds are stored at subzero temperatures. Such seeds are called

TABLE 3.6 *Seed sizes of tree species occurring in British Columbia*

Scientific name	Seeds per gram		Scientific name	Seeds per gram	
	Average	Range		Average	Range
GYMNOSPERMS			*Tsuga heterophylla*	655	416–1119
Abies amabilis	25	18–36	*Tsuga mertensiana*	251	132–458
Abies grandis	50	26–63			
Abies lasiocarpa	85	52–108			
Chamaecyparis nootkatensis	240	145–396	ANGIOSPERMS		
Juniperus scopulorum	60	39–93	*Acer macrophyllum*	7	6–8
Larix laricina	701	463–926	*Alnus rubra*	1468	844–2396
Larix lyallii	313	231–359	*Arbutus menziesii*	570	434–705
Larix occidentalis	302	216–434	*Betula papyrifera*	3040	1344–9083
Picea engelmannii	300	152–709	*Cornus nuttallii*	10	9–13
Picea glauca	405	298–884	*Fraxinus latifolia*	18	13–21
Picea mariana	890	738–1124	*Malus fusca*	119	
Picea sitchensis	465	341–881	*Populus balsamifera* ssp. *balsamifera*	3766	3583–3949
Pinus albicaulis	6	5–7	*Populus balsamifera* ssp. *trichocarpa*	1652	1233–2070
Pinus banksiana	290	156–551	*Populus tremuloides*	8353	5984–10 707
Pinus contorta var. *contorta*	263	225–300	*Prunus emarginata*	15	9–19
Pinus contorta var. *latifolia*	263	225–300	*Quercus garryana*	0.19	0.17–0.22
Pinus flexilis	10	7–14	*Rhamnus purshiana*	27	11–42
Pinus monticola	60	31–70	*Salix amygdaloides*	5720	
Pinus ponderosa	25	15–51	*Salix bebbiana*	5500	
Pseudotsuga menziesii var. *glauca*	85	63–117	*Salix discolor*	no data available	
Pseudotsuga menziesii var. *menziesii*	95	65–100	*Salix exigua*	22 000	
Taxus brevifolia	34	32–36	*Salix lucida* ssp. *lasiandra*	25 000	
Thuja plicata	915	447–1307	*Salix scouleriana*	14 300	

Sources: Stein et al. 1986; Wyckoff and Zasada [1998]; Zasada and Strong [1998]; Zasada et al. [1998].

orthodox in their storage behaviour. Some hardwood seeds do not store well, remaining viable only for several weeks up to 1–2 years. These seeds are called *recalcitrant*. They must be stored at relatively high moisture content (15–40%) and above zero temperatures, and may require other special handling.

3.4.1 Conifer seeds

Collecting conifer seeds

Conifer cones may be collected by climbing (Yeatman and Nieman 1978) or felling trees. Many aerial cone collecting techniques are also available (Camenzind 1990). Aerial methods are much more efficient, especially for species that produce cones in the upper crown, but collection costs are much higher since the use of a helicopter is required. Advantages and disadvantages of various cone collection methods may be found in Camenzind (1990). The choice of method for specific cone collection projects depends both on the crop and the techniques available. Factors to consider include species, crop size, quantity of cones to be collected, site characteristics, the capabilities of each harvesting technique, safety, efficiency, and cost.

For relatively small trees, and where conditions permit, cones and fruits can be collected using a fruit picker with a hydraulic lift. If cone-bearing regions are clearly visible, branches can be shot down with a rifle. Occasionally, cones may be collected from squirrel caches, but it is not recommended because the seeds may be infected with moulds and other pathogens (Sutherland et al. 1987).

Collecting Pacific yew and Rocky Mountain juniper seeds requires strategies different from most other British Columbia conifers. Both Pacific yew and Rocky Mountain juniper are dioecious and bear their fleshy fruits only on female trees.

The fruit of Pacific yew, which ripens in late summer or autumn, consists of a red, fleshy, cup-like aril bearing a single hard seed. To prevent losses to birds, yew fruits should be picked from the branches by hand as soon as they are ripe (Rudolf 1974).

The scales of the female flowers of Rocky Mountain juniper become fleshy and fuse to form small, indehiscent strobili commonly called "berries." Immature berries are green; ripe berries are blue and covered with a white, waxy bloom. The fruit coat of Rocky Mountain juniper is thin and resinous. Juniper berries are usually collected in the fall by stripping or picking by hand directly into bags or baskets, or by shaking or flailing the fruits from the plant onto a canvas spread on the ground (Johnsen and Alexander 1974).

With all collection methods, safety precautions must be rigorously maintained. Safety belts and straps must be checked at least twice each day. Tools such as pruning poles and cone rakes should not be carried while the tree is being climbed. For aerial collections, the helicopter company must be certified, and the pilots appropriately qualified. Aerial collection operations in British Columbia are subject to Workers' Compensation Board regulations; make sure that you have access to current regulations appropriate for the area, and confer with persons experienced in cone collection operations.

Extracting conifer seeds

Serotinous cones such as black spruce may require a period of high temperature to open the cone scales, and sometimes may need multiple extraction cycles, as for example, the procedure used by Haavisto et al. (1988):

1. soak cones in lukewarm water for 2 hours,
2. oven-dry cones at 40°C for 20–22 hours, and
3. tumble cones in a revolving screened drum for 30 minutes.

Using this procedure, an average of eight seeds per cone still remained after the 16th cycle (average seeds/cone = 85).

Note that the application of this procedure and the ones that follow will depend on the degree of serotiny of black spruce cones (see Section 3.5.1).

Mosseler (1992) used only two seed-extraction cycles (SEC) to remove most of the seeds from black spruce cones. The first SEC consisted of oven drying at 50°C for 24 hours. A second SEC was conducted after a 1-hour water soaking treatment, which was followed by drying at 50°C for 24 hours. Few seeds remained in the cones following this extraction procedure, and no further attempt was made to retrieve the remaining seeds. Seeds were counted with an electronic counter and were judged to be filled if they sank in 95% ethanol. Verification of ethanol separation was made by cutting sample seeds from the filled seed fraction, and crushing seeds from the empty fraction.

Although multiple extraction cycles were also used, the method used by Caron and Powell (1989a) differs significantly from the previous two, in that the black spruce seeds are not heated during extraction. Instead, after the cones were shaken individually in a covered jar to dislodge seeds, the remaining seeds were extracted with forceps. This shaking and seed-extraction step was repeated two or three times until all seeds were extracted. Cone scales were separated into three general categories (basal, central, and apical) before being counted. Central scales, which spread apart considerably on cone drying to permit easy release of seeds, were considered potentially seed bearing (fertile). The extracted seeds were separated into filled and empty seeds by alcohol flotation (95% ethanol) after dewinging. X-ray analysis (see Section 7.2.6) indicated that 98.6% of the seeds that sank contained well-developed megagametophyte tissue and a fully developed embryo, whereas 92.6% of those that floated were empty or had a rudimentary embryo. Empty and filled seeds were counted and weighed to the nearest 0.1 mg. Cones (with seed wings) were dried in a forced-draft oven at 100°C for 48 hours and weighed to the nearest 0.1 mg.

In jack pine, which is a predominantly serotinous species, individual cones were dipped in boiling water for up to 30 seconds to break the resinous bonds between cone scales (Rudolph et al. 1986). The cones were dried in a circulating oven at 55°C until they were fully open, after which the cone scales were removed and the seeds were extracted by hand.

Seeds of most conifers (Douglas-fir, larch, western redcedar, western hemlock, etc.) are obtained by drying cones to open them, shaking out the seeds, separating the seeds from cone scales and debris, then loosening the seed wings, and finally separating clean full seeds from wings, dust, empty seeds, and other small particles. It may be advantageous to run closed cones over sorting tables or screens to remove foliage and debris before the cones open. On freshly picked cones of many species (e.g., *Abies*), pitch is soft and sticky. Chunks of pitch that become attached to extracted seeds may be extremely difficult to remove. Therefore, true firs should not be heated, but left under cool, dry conditions on trays to disintegrate naturally. Most other conifer species require only good ventilation and slight heating for several days to open the cones. Small lots of cones can be dried by improvised means in a well-vented laboratory oven with a circulating fan, over a hot-air register or radiator, or similar location.

Cones should be shaken or slowly tumbled to extract the seeds from the opened cones. Small-lot collections of seeds can be efficiently extracted using a multiple compartment tumbler-drier (Leadem and Edwards 1984). Although some wings are loosened during tumbling and preliminary cleaning, many conifer seeds must be dewinged. Wings of most pines and spruces separate readily from their seeds; the wings are hygroscopic, so slight misting can facilitate their removal. For Douglas-fir, larch, and true firs, wings can be gently broken. Wings cannot be removed from western redcedar or yellow-cedar without damaging the seeds.

Wings can be removed from small quantities of seeds by rubbing the seeds between the hands or against a screen or roughened surface. The same principle is employed for larger quantities by gently tumbling dry or wetted seeds in a rotating container such as a cement mixer. Loosened wings, small particles, and dust are removed from good seed in final cleaning. Small lots may be effectively cleaned using a laboratory aspirator (Edwards 1979) or by flotation in water.

The seeds of Pacific yew may be extracted by macerating the fleshy "berries" in water and floating off the pulp and empty seed (Rudolf 1974). Alternatively, the fruits can be soaked for 4–5 days in warm water, then rubbed over screens and washed thoroughly to float off light seeds. The viability of yew seeds can be maintained for 5–6 years if, just after extraction, they are dried at room temperatures for 1–2 weeks, and then stored in sealed containers at 2–5°C.

After twigs, leaves, and other debris have been removed with a fanning mill (air separation combined with screens), Rocky Mountain juniper seeds can be extracted by running the fruit through a macerator with water and floating away the pulp and empty seeds (Johnsen and Alexander 1974). Dried fruits should be soaked in water for several hours before macerating. Seeds should then be dried to less than 10% moisture content (mc) and stored between –6 and +5°C.

For additional information on conifer seed collection, processing, testing, and storage, refer to Stein et al. (1974), Edwards (1982), Eremko et al. (1989), and Leadem et al. (1990).

3.4.2 Hardwood seeds

Hardwoods are more variable than conifers in the time of flowering, seed maturation and dispersal, the type of seed-bearing structures (fruits), and the number of seeds per fruit (Tables 3.1, 3.2, 3.5). Many hardwoods are dioecious; in species such as ash, aspen, willow, and cottonwood, seeds are only produced on female trees. Since the fruits of species such as maple or ash contain only one seed, collection of hardwood seeds may be more labour intensive. For convenience of discussion, the maturation, collection, and processing of hardwood seeds is discussed by fruit type.

*Samaras (*Acer, Fraxinus*)*

Bigleaf maple seeds are double samaras, which turn from green to reddish brown when ripe. The pericarp has a dry, wrinkled appearance when fully mature, and the surface is covered with dense, reddish-brown pubescence. Within the pericarp is an embryo with associated seed coats, but there is no endosperm. Seed collection may begin when the *Acer* samaras are fully ripened and the wing and pericarp have turned tan or brown (Zasada and Strong [1998]). *Acer* seeds may be picked from standing trees or collected by shaking or whipping the trees and collecting the samaras on sheets of canvas or plastic spread on the ground. Samaras may also be collected from trees recently felled in logging operations, and sometimes gathered from the surface of water in pools or streams.

Bigleaf maple seeds should be collected before the fall rains. Once the fall rains start, seed moisture content (MC) may increase from 7 to 35% (dry weight basis) to as high as 50%. If bigleaf maple seeds remain attached to the tree, they may germinate (Zasada 1991). Moisture also affects the longevity of bigleaf maple seeds, which apparently can exhibit either orthodox or recalcitrant seed properties (Zasada et al. 1990; J. Zasada, unpublished data). The significance of collecting before or after the start of fall rains is that bigleaf maple seeds with low MC behave more like orthodox seeds, while seeds collected at high mc have characteristics similar to recalcitrant seeds. The pubescent pericarp may play an important role in the moisture content of the samaras.

Ash fruits occur in clusters of one-seeded samaras, and are collected in fall when their colour has faded from green to yellow or brown (Bonner 1974). Another good index of maturity is the presence of a firm, crisp, white, fully elongated seed within the samara. The clusters can be picked by hand or with pruners and seed hooks. Fully dried samaras may be shaken or whipped from branches of standing trees onto sheets spread on the ground.

After collection, leaves and other debris can be removed by hand-stripping, screening, or using a fanning mill. Since the pubescence on the pericarp can be very irritating to the nose and skin, a face mask and rubber gloves should be used when working for extended periods with bigleaf maple seeds. Maple seeds generally are not extracted from the samaras following collection. However, dewinging reduces weight and bulk for storage, since wings account for about 15–20% of samara weight (Zasada and Strong [1998]). Empty samaras can be removed readily on a gravity table.

Fraxinus samaras should be spread in shallow layers for complete drying, especially when collected early (Bonner 1974). Dried clusters may be broken apart by hand, by flailing sacks of clusters, or by running fruits through a macerator dry. Stems and other debris can then be removed by fanning or with air-screen cleaners.

*Catkins (*Alnus, Betula, Populus, Salix*)*

Birch catkins should be collected while strobiles are still green enough to hold together, or immediately after a rain to keep them from shattering (Brinkman 1974a). In *Populus* and *Salix*, catkins should be collected as close to the time of seed dispersal as possible (Wyckoff and Zasada [1998]; Zasada et al. [1998]). Timing of collection can be based on catkin colour (which changes from green to yellow or yellow-brown) and the condition of the capsule. It is often best to wait until a few capsules start to split (Figure 3.11, stage b) and then collect catkins from the plant, since this usually results in the most rapid opening and efficient seed extraction. Note that insect-damaged capsules may appear to be dispersing seeds, but are often still immature. Once capsules begin to open, the rate of seed dispersal is determined by weather conditions; under warm, dry, windy conditions all seeds may be dispersed within a few days.

If only limited numbers of seeds are needed, branches with attached, immature catkins of *Populus*

and *Salix* can be collected and ripened in a greenhouse or controlled environment (Wyckoff and Zasada [1998]; Zasada et al. [1998]). Catkins must be handled carefully after they have been removed from the tree. During transport catkins should be loosely packed in paper bags to allow for drying. Catkins placed in a warm dry spot will open in a few days, and seeds can be collected as the capsules open.

Since alder catkins do not disintegrate at maturity, they may be collected from standing or recently felled trees as soon as the bracts (scales) start to separate on the earliest-ripening strobiles.

After collection, catkins from *Betula papyrifera* can be air dried on newspapers at room temperature (20–25°C), and the achenes separated from catkin bracts using a series of standard sieves, or with an air-driven seed blower (Bevington 1986). Seed samples can then be stored dry in sealed containers at -23°C until used.

Populus catkins should be spread out in thin layers in pans or on screens at room temperature (Wyckoff and Zasada [1998]). Seeds will be shed in 1–5 days, depending on the ripeness of the catkin. Seeds can be extracted from the catkins with a shop-type vacuum cleaner with a clean cloth bag substituted for the dust bag. *Populus* seeds can be freed from their cotton by tumbling the seeds in a rotating drum or a stream of relatively high-pressure air. For small quantities of seeds, the uncleaned seeds can be placed between two soil sieves and a high velocity air stream applied to tumble the seeds in the container. Seeds should be extracted and placed in subfreezing storage (-5 to -24°C) as soon as possible, since seeds stored at 0–5°C lose viability quickly. Storage with a desiccant appears to provide long-term benefit for *Populus* seeds (Wyckoff and Zasada [1998]).

Salix catkins should not be left at ambient temperatures, and seeds should be extracted and stored

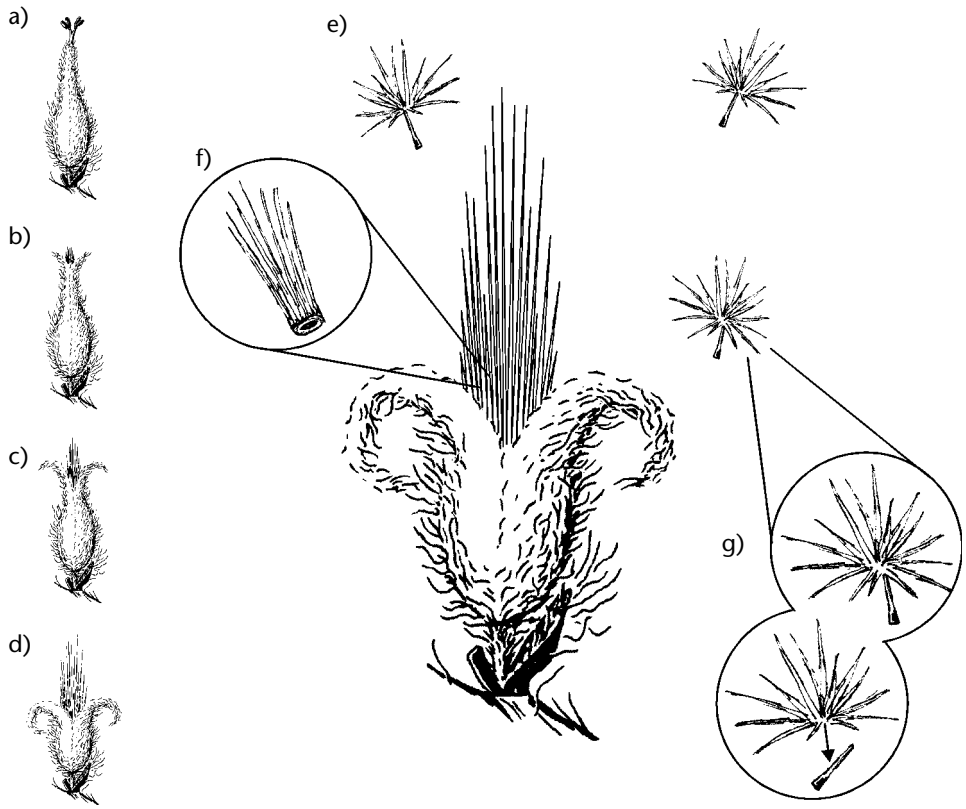


FIGURE 3.11 Salix *capsules at various stages of opening (a-e) and the dispersal unit at various stages (f,g) (Zasada et al. [1998]). (f) shows hairs while still in the capsule; (g) shows hairs fully deployed and separating from the seed. Seeds should be collected when capsules start to split (b).*

at low temperatures as soon as possible (Simak 1980). The seeds should be separated from the cotton to reduce bulk, and because storage with the cotton may reduce viability (Simak 1980). To clean small- to medium-sized lots, place catkins in a single layer in screen-covered boxes in a relatively warm, dry area (20–25°C, 25–35% relative humidity), with good air circulation (Zasada et al. [1998]). If capsules are beginning to open when collected, opening will be completed in 2–3 days. The seeds separate easily from the cotton if the catkins and the cotton containing the seeds are placed in a container, so the material can be blown in an air stream or tumbled in a cement mixer. Seeds can be separated from coarser and finer residue by passing them through a screen or sieve. At this time seed mc should be close to the 6–10% recommended for storage (Simak 1980). To maintain maximum viability, seeds should be placed in sealed containers and stored between -5 and -40°C.

Note that the seed quality of both *Salix* and *Populus* can be graded to a certain degree by passing seeds through a nest of soil sieves. In general, the largest and best seeds will be found on larger sieve openings (J. Zasada, pers. comm., 1996).

Alder strobiles will open after being exposed in drying racks in a well-ventilated room for several weeks at ambient air temperature (Schopmeyer 1974). They can be opened in a shorter time by drying them in a kiln at 27–38°C. Most seeds will fall out of the strobiles during the drying process; however, the remaining seeds may be extracted by shaking or tumbling if necessary.

***Nuts* (Quercus garryana)**

Garry oak acorns are brown when they ripen in late summer and early fall; they may be collected from the ground, or flailed or shaken from branches onto canvas or plastic sheets (Olson 1974). Garry oak belongs to the white oak group, which is characterized by seeds with little or no dormancy, so acorns should be collected soon after they have fallen to retard early germination.

The only processing required before storing or sowing Garry oak acorns is removal of loose cups, twigs, and other debris (Olson 1974). However, the proportion of sound seeds can be increased by removing defective, hollow, and partially consumed acorns, either by flotation or by hand. To retain viability, acorns should be kept under moist, cold conditions. As a member of the white oak group, Garry oak exhibits recalcitrant storage behaviour (Section 3.4), so the mc must not drop below 30–50%.

***Drupes* (Cornus, Prunus, Rhamnus)**

Dogwood fruits are ovoid drupes which ripen in fall. To reduce losses to birds, fruit should be collected as soon as ripe by stripping or shaking from the branches. Ladders may be useful for collecting fruit from taller trees (Brinkman 1974a, 1974b).

Bitter cherry fruits should be collected in late summer or early fall when fully mature and dark red. Fruits are collected by hand-stripping, or by spreading sheets of suitable material under trees to catch the natural fall or fruits shaken off the trees (Grisez 1974). Fruit may be carried in bags, but boxes or baskets provide better protection against bruising and spoilage.

Cascara fruits should be picked in late summer or fall. The fruits are relished by birds so they should be harvested about 2 weeks before they are fully ripe (Hubbard 1974).

To extract seeds of fleshy fruits, most species can be macerated in a blender. Maceration can be facilitated by softening fruits for 3–7 days in running water (or with daily water changes). The mixture is then placed in water to separate the pulp and empty seeds from the good seeds by flotation. Seeds are thoroughly air dried and placed in sealed containers for storage at 2–5°C.

Dogwood stones can be sown without extracting them from the fruit, but seeds to be stored usually are cleaned to reduce bulk (Brinkman 1974b). If fruits cannot be cleaned soon after collection, they should be spread in shallow layers to prevent excessive heating, although slight fermentation may facilitate removal of the pulp. The stones can be extracted by macerating the fruit in water and allowing the pulp and empty stones to float away. Clean, air-dried stones may be stored in sealed containers at 2–5°C.

For bitter cherry it is usually desirable to clean seeds of all pulp and juice (Grisez 1974). Cleaning is done by macerators with water to float off or screen out the pulp. Small quantities may be cleaned by soaking and rubbing over a screen. Fermentation has been used to soften fruit, but germination may be severely reduced if seeds are allowed to become too warm or to ferment too long.

Cascara fruits can be allowed to decay for a few days to soften the pericarp, but usually fruits are run through a macerator with water soon after collecting, then the pulp is skimmed off (Hubbard 1974).

Berries (*Arbutus menziesii*)

The fruit of arbutus is a berry with a thin, rough, granular skin, which is bright red or orange red when ripe. Berries can be collected from standing trees from October to December (Roy 1974).

Arbutus berries can be dried at room temperature or seeds can be separated from the pulp immediately after being collected. Fresh or dried fruit can be soaked in water in a warm place to soften the pulp. Fruits then can be macerated and the seeds separated from the pulp by flotation. Seeds or uncleaned berries should be thoroughly dried, then stored in airtight containers at 2–5°C (Roy 1974).

Pomes (*Malus fusca*)

The pomes of Pacific crab apple are yellowish to reddish when they ripen in late fall. Ripe crab apples may be collected either by picking the fruit from the tree or by gathering fallen fruit from the ground (Crossley 1974).

Pacific crab apple seeds may be extracted by putting the fruits through a macerator with water, floating off the pulp, and screening out the seeds. Seeds should be dried to less than 10% mc and stored at 2–5°C (Crossley 1974).

3.5 Assessing Factors that Reduce Seed Yields

Seed yields are sometimes lower than expected or predicted and we must identify when or why these losses occur, either to verify the value of predictive equations or to prevent future losses. In this section, we examine the effects of serotiny and predation on seed yields. Seed crop losses may occur due to environmental factors, disease, or animal predation, and can be analyzed using life tables (Figure 3.12). Life tables quantify the magnitude and sources of loss and are helpful in interpreting seed crop failure. Life tables might also be applied to hardwood flower production and seed development.

Age interval (months)	Number cones alive	Mortality factors	Number dying	Percent mortality
0–1	1182	*C. pinus pinus*	37	3.13
		Abortion	27	2.28
		Missing	1	0.08
		Breakage	2	0.17
		Unknown insects	9	0.76
			76	6.42
1–2	1106	Abortion	13	1.10
		Shoot borer	23	1.95
		Missing	1	0.08
			37	3.13
2–5	1069	Abortion	122	10.32
		Missing	5	0.42
		Squirrel	4	0.43
			131	11.08
Conelets remaining	938	Total mortality	244	20.54

FIGURE 3.12 *Partial life table for 1981 jack pine conelet crop, Oneida County, Wisconsin (adapted from Rauf et al. 1985). Life tables quantify the magnitude and sources of loss and are helpful in interpreting seed crop failure.*

3.5.1 Assessing serotiny

Some conifers do not shed their seeds when they mature in the fall, and instead may retain their seeds in the cones for several years. This is termed *serotiny*, but is sometimes called *canopy banking* (as opposed to seed banking in the soil). Serotinous species retain their seeds in tightly closed cones until high temperatures (such as those achieved in a forest fire) open the cones. The degree of serotiny appears to depend on such factors as the frequency of fire, the local climate, and hybridization between interior populations that are predominantly serotinous and coastal populations that are not. Serotiny has great silvicultural significance because large quantities of seeds are potentially available for release after fires or harvesting.

In British Columbia, coastal lodgepole pine is primarily non-serotinous, whereas interior lodgepole pine usually bears serotinous cones (Eremko et al. 1989). In both varieties, cones remain on the trees for many years, but freshly ripened cones have the highest number of viable seeds. Jack pine are serotinous over most of their range, although southern sources tend to be non-serotinous. Black spruce cones are semi-serotinous; the cones remain on the tree and the seeds are viable for several years (Safford 1974), and sometimes as long as 15 years (J. Zasada, pers. comm., 1997).

To estimate the quantity and quality of seeds available for regeneration, it may be necessary to assess the age of serotinous cones. Eremko et al. (1989) provide photographic examples of lodgepole pine cones in different age classes, and recommend that, for lodgepole pine, only cones in classes I and II (i.e., less than 5 years old) be collected. The cones should be only partially weathered and completely closed.

Viability of seeds in serotinous cones of harvested trees can decline rapidly, and older cones present in the slash may have to be discounted as a source for natural regeneration of a site. Ackerman (1966) found that 3 years after logging there was a substantial decrease in the germination percentage of seeds. To conduct his study Ackerman devised scales to classify the degree of serotiny and degree of weathering of lodgepole pine cones present in logging slash:

Classes of resin-bond rupture

1. fully open cone	scales free over 81–100% of cone surface
2. partly open	scales free over 21–80% of cone surface
3. closed	scales free over 0–20% of cone surface

Classes of weathering as index of age

1–3 yr	no evidence of weathering
2–7 yr	weathered over 5–25% of surface
6–13 yr	weathered over 26–50% of surface
12–20 yr	weathered over 51–75% of surface
16+ yr	weathered over entire surface

3.5.2 Assessing predation

Seeds represent an excellent food source because of their stored reserves, thus mature seed crops are attractive to insects, birds, squirrels, or other animals. This section primarily describes predation of immature seeds (pre-dispersal); for a detailed discussion of the predation of mature seeds, see Section 5.

During excellent seed years there are usually more than enough seeds to support both animal predation and natural regeneration. In moderate years, however, predation can present a problem. Since predator populations usually lag a year or so behind abundant seed crops, a mast year is often followed by a poor year with higher predator populations. Insect and disease damage to seeds and cones may range from moderate to severe, and sometimes can result in the loss of an entire seed crop (Miller et al. 1984; Schmid et al. 1984). Depending on the type of insect or disease, the attack may occur any time from bud initiation to final seed development. Damage may result in cone or seed abortion or in partial or complete destruction of cones or seeds (Mattson 1978). Effects are sometimes indirect, for example, insects or disease may cause the premature opening of cones so that seeds are shed before they are fully developed.

Insect predation can alter cone crop phenology (Rauf et al. 1985) and seed dispersal, and may cause conelet and cone mortality. Seed losses due to insect predation can be determined by dissecting the cones and examining cone length, width, and the number of sound, hollow, and insect-damaged seeds (Schmid et al. 1984). The percentage of seeds damaged in each cone may vary, depending upon the insect species.

In areas where squirrel predation can have a major impact on natural seed production, it is advisable to

collect cones early, but only if seeds can be ripened sufficiently under artificial conditions. Hurly et al. (1987) found that intensive harvesting by red squirrels began in early September, and most caching occurred in late September and October. Early in this period most cones cut were eaten rather than cached. Caches are easily found; cones can be recovered from the caches within 4 weeks following the peak of caching behaviour.

More information on cone and seed insects is available in Hedlin (1974), Hedlin et al. (1980), Ruth (1980), and Ruth et al. (1982).

Microbial diseases may also be considered seed predation, and substantial cone and seed losses due to disease occur each year. For further information on cone and seed diseases of North American conifers, refer to Sutherland et al. (1987) and Ruth et al. (1982).

3.5.3 Using X-ray analysis to determine causes of loss

Seed X-rays are a quick and effective way to analyze seed production, but they depend upon the use of expensive X-ray equipment. If such equipment is available, X-radiography can provide non-destructive measurements of the number of filled, immature, and empty seeds, as well as the numbers of seeds which have been damaged or attacked by insects (Figure 3.13). In research studies, comparison of seeds to their X-ray images facilitates the efficient removal of empty seeds. For detailed procedures, see Section 7.2.6 and Leadem (1984).

3.6 Experimental Design

"Cheshire Puss," she began, "would you tell me, please, which way I ought to go from here?"
"That depends a good deal on where you want to go to," said the cat.
(Lewis Carroll "Alice in Wonderland")

A study design is a plan for obtaining the maximum amount of information from available resources (Sit 1995). A good design should begin with clear, well-defined objectives. Three general objectives of natural seed production studies are:

- estimation (e.g., how many seeds per cone?)
- modelling (e.g., what is the relationship between seed production and stand, tree, and crown characteristics?)
- comparison (e.g., is seed morphology the same in cliff and swamp areas?)

3.6.1 Estimation studies

To estimate a parameter such as the average number of seeds per cone or the total number of seeds in a plot, proper sampling design must be considered to ensure that the estimates are unbiased. Many sampling designs can be used, such as simple random sampling, cluster sampling, stratified sampling, and multistage sampling. For detailed discussions on these and other sampling schemes, refer to Cochran (1977), Thompson (1992), and Buckland et al. (1993).

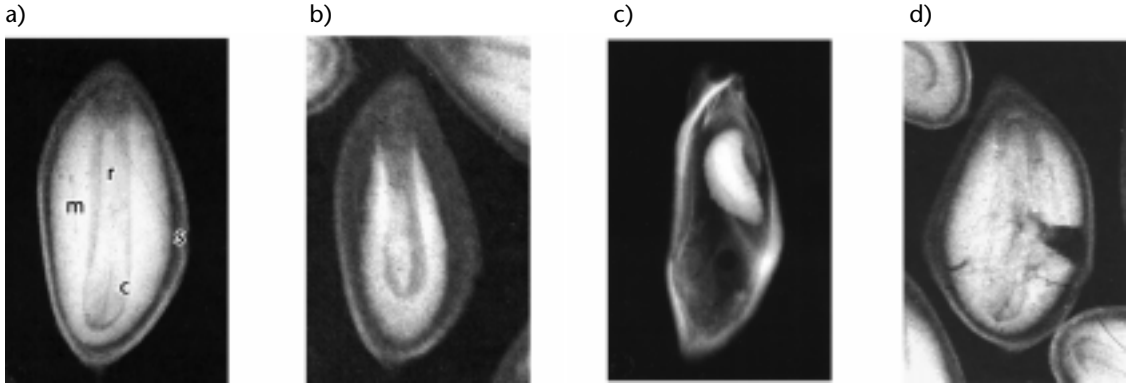


FIGURE 3.13 *X-rays of tree seeds (Leadem 1996). X-rays are used to determine whether seeds are fully developed, damaged, or have been attacked by insects. (a) mature seed: c = cotyledon, m = megagametophyte, r = radicle, s = seed coat; (b) immature seed; (c) insect larva; (d) damaged seed.*

Regardless of which sampling design is chosen, sampling should be done through a random mechanism. This will ensure that no systematic bias will be introduced to the data. Sometimes, due to convenience or convention, an investigator may subjectively select samples that are considered typical for the population of interest. These samples, called representative and judgement samples, are discouraged because they are subject to personal bias and their statistical properties are unknown. Systematic samples are often taken because of their ease of execution. However, they can be unreliable, especially when the sampling scheme coincides with an underlying pattern in the sampling population. It is best not to consider systematic samples for estimation.

To capture the variability in the population of interest, the sampling design must provide an adequate sample size. The sample size depends on the variability in the population, the accuracy desired, and the cost. You may want to consider stratifying the collection of samples (e.g., collecting from different levels of the crown of a tree) to reduce variation and better understand effects of position (vertical and horizontal). For estimation type studies, the sample size can be determined using confidence interval methods. See Section 3.1.3 for a discussion and an example of sample size determination methods using confidence intervals.

3.6.2 Modelling studies

To sample for modelling, the sampling guidelines discussed above should be followed. All variables involved in the model must be sampled from the same sampling points. For example, if you want to relate the number of seeds per cone with the number of exposed seeds in the cone half-face, then the total seeds per cone and the seeds in the half-face must be determined from the same cone.

To model a relationship, there must be enough data to capture the relationship between the variables. A general rule is to have at least 10 data points per parameter involved in the model. For example, a straight line model,

$$Y = a + bX,$$

has two parameters, Y-intercept (a) and slope (b), and requires at least 20 data points. A logistic model,

$$Y = \frac{a}{1 + e^{b-cX}},$$

has three parameters (a, b, and c) and requires at least 30 data points.

The data collected should also cover the full range of interest. For example, suppose you want to model the relationship between cone production and accumulated growing degree-days (GDD). If you want to use the model to predict cone production for 600–1300 GDD, then the data you use in developing the model must span the range 600–1300 GDD. The resulting model would only be suitable for predictions within this range; extrapolations beyond the range would be unreliable.

In general, more data points are needed for complex relationships than for simple relationships.

3.6.3 Comparative studies

In contrast to sampling for estimation and modelling, comparative studies require an experimental design. In a comparative experiment, treatments are randomly assigned to a number of experimental units (the smallest collection of the experimental material to which a treatment is applied). If you wish to compare seed morphology in two different habitats (e.g., cliff and swamp), five sites each can be selected randomly from all cliffs and swamps within the population of interest. Within each site, 10 trees can be selected for cone measurement. In this example, a site is the experimental unit; a tree or a cone is a subsample.

Comparisons based on a single application of the treatments are unreliable because variations are expected between experimental materials. Differences between a cliff site and a swamp site could be due to differences in the habitat, or to natural variation from site to site, or both. The only way to distinguish the possible causes of variation is to replicate the treatments.

Replication of a treatment is an independent observation of the treatment. The number of replications is the number of experimental units to which a treatment is assigned. Replication should not be confused with subsamples, which are multiple measurements of a single treatment. In the cliff/swamp example, each treatment is replicated five times. The 10 trees within each site are subsamples. Pseudoreplication occurs when replication is claimed when in fact there is none. Pseudoreplication usually leads

to underestimation of the variability in the data. See Bergerud (1988) for additional discussion of pseudoreplication.

The number of replications necessary for a study depends on the variability in the data, the size of difference you wish to detect, the significance level desired, and the desired statistical power. Power analysis is the computation of statistical power for an experimental design, and should be carried out before the experiment to determine the amount of replication required. For more discussion on the use of power analysis for sample size determination, see Cohen (1977) and Nemec (1991).

Random assignment of the treatments to the experimental material is also essential to sound experimentation. Randomization assures that no systematic bias is introduced to the experiment, and the natural variation is approximately the same within each treatment group. Sometimes random assignment of the treatments to the experimental material is not possible. In the cliff/swamp example, it is not possible for the experimenter to assign a cliff or a swamp to a particular location; an area is a cliff or a swamp before the experiment is even conceived. In this case, to satisfy the randomization criteria, cliff and swamp sites must be randomly selected from all cliff and swamp sites within the population of interest. Subsamples for measurements must also be randomly chosen within each experimental unit.

The design of a comparative experiment depends largely on the treatments to be compared, the experimental material available, and the type of data to be collected. Common experimental designs employed in seed production studies include completely randomized design, factorial designs, and randomized block design. For discussions on these and other experimental designs, see Sit (1995).

It is vital that the sampling design and experimental design optimize all essential factors of the study. Researchers should discuss their designs and analysis plans with a statistician before implement-ing a study to ensure that all relevant factors have been considered.

3.7 Data Analysis

The success of an experiment requires both a well-designed plan and an appropriate analysis method, because the two are closely related. The method of analysis depends on the design plan, while the design plan is strongly influenced by the analysis method deemed to be the most suitable for the data. The analysis method should conform with the design of the study, the nature of the data, and the study objectives.

3.7.1 Estimation studies

If the objective of a study is sampling for estimation, then care must be taken to ensure that the formulae for computing mean, total, and standard error are appropriate for the chosen sampling scheme. A common mistake is to use formulae for simple random sampling design in more complex designs, which results in underestimation of the standard error of the estimate. That is, the estimate would appear more reliable than it really is. Nemec (1993) provides an example that illustrates the consequences of using simple random sampling formulae for data collected from cluster sampling.

3.7.2 Modelling studies

When the objective is to sample for modelling, then regression and correlation are typical analysis methods. Depending on the relationship between the variables of interest, linear or nonlinear regression may be required. If the goal is to determine the best set of variables for predicting a relationship, then stepwise regression can be used to systematically eliminate any unnecessary variables.

Regression assumes that the residuals (the difference between the observed data and the predicted values) are normally distributed, with a mean equal to zero and a constant standard deviation. The normal distribution of the residuals can be checked using a normal probability plot on the residuals. An apparently straight line indicates that the residuals are approximately normally distributed. Regression also assumes that the residuals are: a) independent of the values in the explanatory variables (x-variables), and b) have equal variance for all values of the explanatory variables. The independent and equal variance assumptions can be checked by plotting the residuals against the predicted values derived from the regression model. A random scatter of the points implies that both assumptions are satisfied.

Violation of the normality and equal variance assumptions sometimes can be corrected by

transforming the data using square root, natural log, or exponential functions. Transformation should not be done routinely without first checking the residuals. Keep in mind that the regression assumptions are for the residuals, not for the data. It is possible to have non-normal data, but normal residuals. A common mistake is to examine the data and apply transformation when the data are not normal or have unequal variance.

Regression is a robust procedure against slight departures from normality and equal variance when the data set is large. That is, with a large data set, you can still use regression on the data (without transformation) for slightly non-normal residuals. However, like most statistical procedures, regression is not robust against independence. That is, regression results are invalid if the residuals are dependent (e.g., when large residuals tend to associate with large *x*-values.) Provision for randomization during data collection will ensure that the data, and thus the residuals, are independent.

To assess the goodness of fit of a model to the data, the coefficient of determination, r^2 value, can be calculated. The coefficient of determination represents the proportion of variation in the data explained by the model. The higher the r^2 value, the more variation is accounted for by the model. The r^2 value is directly related to the number of explanatory variables in the model: the more explanatory variables there are, the higher the r^2 value. When comparing several regression models, it is more suitable to use the adjusted coefficient of determination (r^2_{adj}) which is r^2 modified by the number of explanatory variables in the model. A model with large r^2_{adj} is favourable, that is, it explains most of the variation in the data with the minimum number of variables. Rawlings (1988) may be consulted for further information on regression analysis. See also Sit and Poulin-Costello (1994) for additional discussions on nonlinear regression.

If the objective is to assess the strength of the relationship between two variables, then correlation analysis can be used. There are two types of correlation: Pearson product-moment correlation coefficient, r(p), and Spearman's rank order correlation coefficient, r(sp). The Pearson correlation assesses the linear relationship between two variables (see Figures 3.14a and b), and is based on the observed data. Spearman's correlation assesses the monotone relationship between two variables, that is, whether the two variables have a strictly increasing (linearly or nonlinearly) or strictly decreasing relationship (see Figures 3.14c and d). Spearman's correlation is based on the rank order of the data, with tied scores assigned the average of the scores that would have been assigned had no ties occurred.

A correlation coefficient must have a value between +1 and -1. A positive value implies that the two variables increase together; a negative value implies that one variable increases as the other variable decreases. A Pearson correlation coefficient near zero implies there is no linear relationship between the two variables, but the two variables may be related in a nonlinear way (see Figures 3.14c, d, and e). A Spearman's correlation coefficient near zero implies that the two variables do not increase or decrease together, but they may be related in a curvilinear manner (Figure 3.14e).

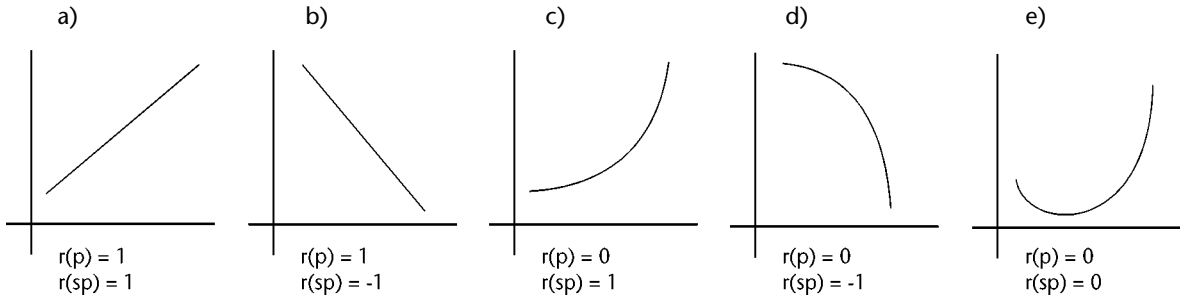


FIGURE 3.14 *Correlation coefficients for hypothetical relationships. r(p): Pearson product-moment correlation coefficient; r(sp): Spearman's rank order correlation coefficient.*

3.7.3 Comparative studies

If the objective is to compare the effects of several treatments on seed production, then analytical methods should be used. The method chosen depends on the nature of the data and the design of the study. For continuous data such as seed weight, seed length, or seed width, methods such as the *t*-test and analysis of variance (ANOVA) *F*-test could be used for analysis. Both the *t*-test and the ANOVA *F*-test assume normally distributed residuals. This assumption can be checked by plotting the residuals in a normal probability plot (see Section 3.7.2). If the residuals are far from normal, then nonparametric procedures such as the Wilcoxon tests could be used. Refer to Sections 3.7, 4.5.2, 6.5, and 7.2.5 for discussions of ANOVA analysis. See Sit (1995) for a detailed discussion of ANOVA.

For discrete data such as seed crop rating or the number of full and empty seeds on a tree, contingency table tests (chi-square test, Sections 5.5 and 8.3.4) or log-linear models could be used. If data are collected on the same units over time and the objective is to assess trend, then repeated measures analysis methods should be considered. See Nemec (1996) for detailed discussions of repeated measures analysis.

3.8 Seed Production Case Studies

Six seed production case studies, taken from the literature, are summarized below. To highlight the design and analysis aspects, the studies are presented in point form. The cautions given at the end of each case emphasize the items that require special attention to ensure that study objectives are met.

CASE STUDY 1: Estimating potential Engelmann spruce seed production on the Fraser Experimental Forest, Colorado (Alexander et al. 1986)

Objectives

- To predict the frequency of good seed crops.
- To relate seed production to stand, tree, and or crown characters (sampling for modelling).

Study Design

1. The sampling was carried out over a long period of time (annually for 15 years).
2. Thirteen permanent sample plots with 10 seed traps were randomly located in each plot.
3. Seed trap contents were collected each fall and again the following spring.
4. Only filled seeds were counted; the response variable was the number of filled seeds per trap.

Data Analysis

1. The sample mean (by plot) was the best estimate of average number of filled seeds per trap.
2. ANOVA was used to test for location and year effects.
3. Seed counts were transformed ($\sqrt{x + 3/8}$) to stabilize variance.
4. Regression was used to relate the number of filled seeds per trap and the total seedfall per trap.
5. Stepwise regression was used to select the best set of stand, tree, and/or crown measures for predicting seed production.
6. Transformation of the data may be considered to correct for heterogeneity of variance before regression; possible transformations are square root and natural log.

Cautions

- Sampling units should be randomly selected from all possible units.
- Seed traps should be randomly located within each sampling unit (sample plot).
- Seeds from several traps should not be bulked.
- If data are collected over a long period of time, check whether the model residuals are independent; and consider using time-series models or repeated measures (incorporating lag variables in the regression).

CASE STUDY 2: Comparative seed morphology of *Thuja occidentalis* (eastern white cedar) from upland and lowland sites (Briand et al. 1992)

Objectives

- To test whether a relationship exists between seed morphology and the habitat where seeds are produced (cliff and swamp).
- To explain the greater root plasticity among upland seedlings.

Study Design

1. This is a one-factor completely randomized design with subsamples.
2. Three cliff sites and three swamps were randomly selected.
3. Ten trees were sampled at each site; five cones were collected from each tree for measurements.
4. Responses included total number of seeds, number of fully developed seeds per cone, seed fresh weight, seed length and width, embryo area length and width, and right wing length and width.

Data Analysis

1. ANOVA was used to compare the responses of the two habitats (at significance level 0.05).
2. Sequential Bonferroni technique was used to adjust the α-probability level for simultaneous comparisons to reduce the risk of Type I error.
3. Satterthwaite's approximation (Zar 1984) was used to compute 95% confidence intervals based on the *t*-distribution.
4. Pearson product-moment correlation coefficients were computed between all characters.

Cautions

- Since cliff and swamp could not be assigned to an area, sites were randomly selected from all cliffs and swamps within the population of interest.
- Be careful when identifying the experimental unit. In this example, an individual site is the experimental unit, not tree, or cone.
- The trees in the two habitats should be as similar as possible to avoid confounding the habitat and tree characteristic factors (e.g., cliff sites had older trees).
- If the two habitats had trees of different ages, age could be used as a covariate in the analysis of covariance, provided that the covariate (age) is not affected by the factor of interest (in this case, habitat).

CASE STUDY 3: Cone size and seed yield in young *Picea mariana* trees (Caron and Powell 1989a)

Objectives

- To investigate the variation in cone size, seed yield per cone, and seed weight from cones collected in 4 plantations in three consecutive years.
- To determine the correlation between cone size, seed yield per cone, and seed weight.
- To examine the relationship between pollen abundance and filled seeds per cone.

Study Design

1. Five plantations (8, 10, 12, 14, and 16 years from seed in 1980), located in northwestern New Brunswick, were used in the study.
2. Two study areas were selected within each plantation; trees were randomly selected for measurement.
3. Responses included cone length, cone weight, total scales per cone, potential filled seeds per cone, total seeds per cone, total filled seeds per cone, seed efficiency, weight of 1000 filled seeds, and weight of 1000 empty seeds.

Data Analysis

1. Correlation was used to assess the relationships of the nine response measures.
2. Regression was used to relate the number of filled seeds per cone to the number of pollen cones per tree; logarithmic transformation was used on the response variables to correct for unequal variance.

Cautions

- Correlation can be used to assess the relationship between two variables, but Pearson correlation can assess only linear relationships. It is possible that two variables are nonlinearly related and the correlation coefficient is near zero.
- A variable that shows a high correlation to seed yield per cone may not be the best predictor for seed yield; another variable that is nonlinearly related with seed yield may be a better predictor.
- Always plot the data in a scatter plot.

CASE STUDY 4: Prediction equations for black spruce seed production and dispersal in northern Ontario (Payandeh and Haavisto 1982)

Objective

- To use nonlinear regression to relate the number of black spruce seeds per cone with cone age and crown class.

Study Design

1. Data on seed production (number of seeds per cone and cone age in years) were collected from black spruce in three crown classes: dominant, codominant, and intermediate.
2. Two sets of data on seed dispersal across the stripcuts were also available for modelling.

Data Analysis

1. A simple exponential decay function, $Y = B_1 e^{-B_2 X}$, was fitted to the data to relate the total number of seeds per cone (Y) with cone age (X) for each crown class.
2. An inverse sigmoidal function,

$$Y = B_0 - B_1(1 - e^{-B_2 X})^{B_3},$$

was fitted to the data to relate seed viability (Y) with seed cone age (X).

3. An exponential decay-exponential model,

$$Y = B_1 X_1^{B_2 e^{-B_3 X_2}} + B_4 X_1^{B_5 e^{-B_6/X_2}},$$

was developed to relate estimated seedfall per hectare (Y) with strip width (X_1) and distance from stand edge (X_2).

Cautions

- Enough points are needed to cover the entire X-range.
- Know the form of the equation, and the derivatives with respect to the unknown parameters, and an estimate of the parameters (starting point for iteration).
- Models should be compared based on adjusted r^2.
- Do not extrapolate results from the fitted models beyond the range of the original data.

CASE STUDY 5: Estimating sound seeds per cone in white spruce (Fogal and Alemdag 1989)

Objectives

- To determine whether the number of filled seeds per cone half-face is a valid indicator of the total number of seeds per cone.
- To determine the relationship between the number of filled seeds per cone and cone length and diameter.
- To determine whether the relationship is the same across time and location.

Study Design

1. Cone data were collected from three white spruce plantations in 1982 and 1984.
2. Seed counts were made on 10 cones from each of 20 trees at each location.
3. Measurements included cone length and maximum diameter, number of sound seeds per section on one cone half-face, and number of sound seeds per cone.

Data Analysis

1. The mean and coefficient of variation were calculated for each of the four variables for each location and crop year.
2. ANOVA was use to compare locations and years based on the number of seeds per cone.
3. Scattergrams were prepared for each location and year to visually assess possible relationships between number of sound seeds per cone and number of sound seeds per section, cone length, and diameter.
4. Multiple regression was used to relate the number of sound seeds per cone with the following independent variables: number of sound seeds per section, cone length, and cone diameter. Eight models were fitted to the data.

Cautions

- Use adjusted r^2 to compare models, not r^2.

CASE STUDY 6: Consistency of cone production in individual red pine (*Pinus resinosa*) (Stiell 1988)

Objectives

- To compare production by stand and by individual trees at two dates (1970 and 1984).
- To relate cone production to stem diameter and subsequent diameter growth.

Study Design

1. Data were collected from an 18-year-old red pine plantation that was established as a spacing trial.
2. Permanent sample plots were established for each of six spacings.
3. Cone counts were made on mechanically selected, numbered trees in 1970 and 1984.

Data Analysis

1. Linear regression with square root transformation was used to relate crop size to tree size at both dates, and to relate 1984 crop size to 1970 crop size.
2. Potential cone production was approximated using the sum of both mature and aborted cones.
3. Relationship between 1970 crop size to 1970–1972 basal area growth was also analyzed.

Caution

- Use adjusted r^2 to compare models.

SECTION 4 SEED DISPERSAL

Lo! sweetened with the summer light,
the full-juiced apple, waxing over-mellow,
Drops in a silent autumn night.
(Alfred, Lord Tennyson "The Lotus-Eaters")

4.1 Background

Seeds may have many potential fates, as shown by the descriptive model (Figure 4.1), which follows the dispersal of the seeds of eastern redcedar. Seed dispersal refers to the movement of seeds or fruit, beginning with seed release from the parent tree and ending with the seed coming to rest at the spot where it will eventually germinate or die. While trees remain stationary over most of their life span, their seeds and pollen can travel great distances during dispersal. Effective seed dispersal, by enhancing the capacity of individuals to colonize new locations, may determine the survival of the next generation of plants.

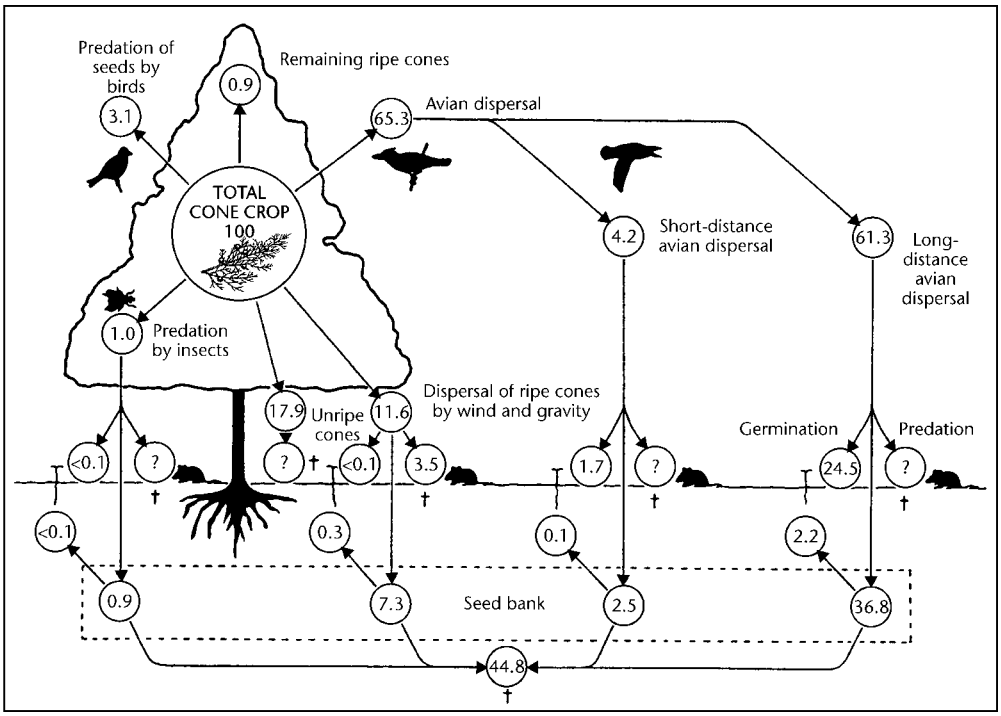


FIGURE 4.1 *A descriptive model of eastern redcedar (*Juniperus virginiana *L.) cone-crop dispersal from June through May of the following year (Holthuijzen et al. 1987). Numbers are percentages of the total cone crop and are means for four sample trees.*

There are six major types of seed dispersal: wind dispersal, vertebrate dispersal (ingestion), ant dispersal, ballistic dispersal (where the seeds are ejected explosively from a seed pod), adhesive dispersal (seeds that have hooks, barbs, or sticky substances that cling to fur or feathers), and unassisted dispersal (where no obvious dispersal adaptation is present) (Hughes et al. 1994). Seeds and fruits of British Columbia tree species are usually wind dispersed, vertebrate dispersed (ingestion), or unassisted (gravity). A second form of vertebrate dispersal is also found in British Columbia, where highly nutritious, long-lasting seeds are moved to food caches and stored for later use.

This section will look at primary and secondary dispersal of British Columbia tree species, some methods for quantifying seed dispersal, and typical methods for analyzing the data.

4.1.1 Why study seed dispersal?

Seed dispersal is of interest to plant ecologists and to researchers studying evolution (see Harper 1977). Seed dispersal patterns influence the genetic structure of tree populations, variation in the composition of forest stands, and the success of many silvicultural prescriptions for natural regeneration. Many studies use tree seed dispersal as a starting point, because natural regeneration of a species requires suitable levels of seed production and seed rain (the overall input of seeds per unit area per unit time). Estimates of stand-level seed production and seed rain are often a component of silvicultural system trials.

Questions that might be asked in seed dispersal studies include the following:

- What is the primary seed dispersal mechanism for a species?
- How does the seed dispersal pattern of one species affect establishment of another species? (For example, interspecific competition for germination sites.)
- What is the species composition of the seed rain, and how does it vary from year to year, or from place to place on the landscape?
- What is the spatial distribution of seeds relative to good germination spots, source trees, etc.? Are there differences among species? What are the primary factors affecting the distribution?
- What spatial patterning is exhibited (in terms of uniformity, clustering, linear gradients)?
- What is the temporal distribution of seeds:
  - over a day?
  - over a season?
  - over a year?
  - over several years?
- How does opening size (or any of the other factors that affect dispersal patterns) influence the density and/or distribution of seeds?
- What proportion of dispersed seeds undergo secondary dispersal?
- Which species exhibit secondary dispersal?
- Under what conditions does secondary dispersal occur?
- How do primary and secondary seed dispersal patterns affect what seral communities are likely to become established during primary or secondary succession?
- How do different silvicultural treatments affect the distribution of seeds?

4.1.2 Mechanisms of seed dispersal

Seed dispersal has two phases (Watkinson 1978): primary dispersal, in which seeds travel from the parent tree to the surface of the ground; and secondary dispersal, during which the seeds are moved by one or more agents after hitting the ground.

Primary dispersal may be passive (the seeds fall to the ground because of gravity), generally found in large-seeded species such as Garry oak or Rocky Mountain juniper, or dispersal may be aided by traits that facilitate active seed movement by wind, water, or animals. For seeds of most western conifer species, wind is the primary dispersing mechanism (McCaughey et al. 1986), so seeds of white spruce, subalpine fir, western larch, and western white pine, for example, have aerodynamic adaptations such as wings to aid in the dispersal of seeds.

Wind dispersal is also common in northern angiosperm tree species, and many have evolved structures to optimize the distance their seeds are carried. The major categories of wind-dispersed angiosperm fruits are samaras (the self-rotating, winged seeds of most *Acer* and *Fraxinus* spp.), plane-winged seeds (like those produced by *Alnus* spp. and *Betula* spp.), and the light plumed seeds produced by *Populus* and *Salix*.

Many trees and shrubs have brightly coloured, nutritious, fleshy fruits that are consumed by birds and other animals. Among British Columbia tree species, this dispersal mode is used by wild cherries (*Prunus* spp.), hawthorns (*Crataegus* spp.), mountain ash (*Sorbus* spp.), Pacific dogwood, and cascara.

Other trees have large wingless seeds that are often moved to distant microsites by hoarding animals or birds. Several British Columbia tree species, such as Garry oak, yew, and several pine species including whitebark pine, use this dispersal mechanism. Clark's nutcracker (*Nucifraga columbiana*) is the primary dispersal agent of limber pine and whitebark pine seeds. These seeds may be moved long distances to caches under forest litter. Some seed predators (Section 5) inadvertently act as dispersers because many of the hoarded seeds are never consumed and germinate in the caches (Hutchins and Lanner 1982).

Secondary dispersal refers to the movement of seeds after they have fallen to the ground. Secondary dispersal can result in very long-distance seed movement, but more often results in movement over only short distances (Zasada et al. 1992). Even seeds adapted for dispersal by wind or birds can be moved by other dispersal agents before they eventually come to rest. Seeds may be cached by chipmunks, but be redistributed by water during spring runoff or moved by wind across snow.

Water is the agent with the greatest potential for long-distance secondary dispersal of seeds. Tree species found in floodplains often have their seeds dispersed by water, and the movement of seeds on rivers has been observed for many species. Seeds of *Populus* seem to be adapted to this type of dispersal as they readily germinate and root under water (Zasada et al. 1992).

The importance of over-snow dispersal depends on the time of seed dispersal, the quantity of seeds, and the timing of annual snowfall (Dobbs 1976; Matlack 1989). Seeds dispersed before the first winter snow cannot be transported in this manner.

Secondary seed dispersal may result in movement of seeds over a short distance, however, the movement may be very important, such as when seeds are moved to a better microsite than would have resulted from primary dispersal (Zasada et al. 1992). Seeds released during the winter from western hemlock, *Alnus*, and *Betula* can be dispersed by wind across crusted snow, accumulating in large numbers in small depressions.

4.1.3 Timing of seed release

The timing of seed rain for different species can be placed into three categories: (1) species whose seeds mature in summer and disperse during the current growing season; (2) species whose seeds mature in late summer and are dispersed during the dormant season; and (3) species whose seeds mature in late summer, but dispersal is delayed for several to many years (species with serotinous and semi-serotinous cones) (Zasada et al. 1992).

1. Species that disperse seeds in summer include willows, trembling aspen, and balsam poplar. The dispersal period for these species is usually not more than 1 month.
2. Fall and winter seed dispersal occurs in most western conifers, as well as birch, alders, and some willows (McCaughey et al. 1986; Zasada 1986). Timing of seed dispersal from mature conifer cones and alder catkins is determined by the occurrence of weather which dries them, opening the scales and allowing the seeds to be released (Harrington et al. 1994). In general, wet weather keeps cones and catkins closed, and wet weather following dry weather closes catkins, terminating a dispersal period.

   In fall-dispersed seeds, dormancy requirements are satisfied after seeds have reached the ground. In winter- or spring-dispersed seeds, the dormancy requirements may be partially or completely met while the seeds are still attached to the parental tree. In some species this results in germination while seeds are still attached to the tree (e.g., bigleaf maple) (Zasada 1991).
3. Delayed dispersal occurs in several serotinous species, such as black spruce, jack pine, and lodgepole pine (see Section 3.5.1). Black spruce retains its seeds in semi-serotinous cones for many years, and annual seedfall occurs as cones gradually open. Seed dispersal is usually greater in late spring and summer, but some seeds may be dispersed year-round (Zasada 1986). Fire flexes the scales of serotinous cones, thus large quantities of seed may be dispersed immediately after a fire. Timing and length of the dispersal period depends on fire intensity and weather. Most serotinous species have

hairs or wings, adapting them for dispersal by wind (Hughes et al. 1994). Wind dispersal may be enhanced in the immediate post-fire period because fire opens the canopy and clears the ground of obstacles to the passage of wind and seeds (Hughes et al. 1994).

4.1.4 Dispersal distance

The distance a seed travels from its parental source depends on its mode of dissemination—gravity, animals, or wind. Dispersal distance is often expressed as a dispersal curve (Figure 4.2), which is the frequency distribution of dispersed seeds versus the distance from the seed source.

Heavy, nonwinged seeds usually depend on gravity or animals for dispersal. When gravity is the sole means of dispersal, the seeds will remain very close to the parental tree. The distance these seeds travel may depend on the slope, with seeds travelling farther on steep slopes. Many non-winged seeds also rely on animals either as primary or secondary dispersal agents. When seeds are dispersed by animals, the distance the seeds travel from the source can be highly variable.

When seeds are eaten, birds and mammals can defecate or regurgitate the seeds far from their point of origin. Generally, among fruiting species, small seeds are dispersed farther than large seeds (Hoppes 1988). The dispersal distances for ingested seeds are limited by gut passage rates, which vary from a few minutes in frugivorous birds to several months in ungulates (see review by Willson 1993). The direction of seed movement and the local density of seed deposition will be influenced by the behaviour of the particular frugivore (Stiles 1992). Caching of seeds by squirrels and chipmunks also extends the dispersal distance of the seeds. Clark's nutcrackers carry seeds long distances to caches under forest litter, where, if conditions are favourable, seeds may subsequently germinate (Tomback 1981).

Wind-dispersed seeds generally travel farther than animal-dispersed seeds (Willson 1993). For comparative tables of dispersal mechanisms and distances, see Hughes et al. (1994). Species that most efficiently disperse seeds are the willows, aspen, and balsam poplar (Zasada et al. 1992). Observations show that these seeds can be dispersed in relatively large quantities to

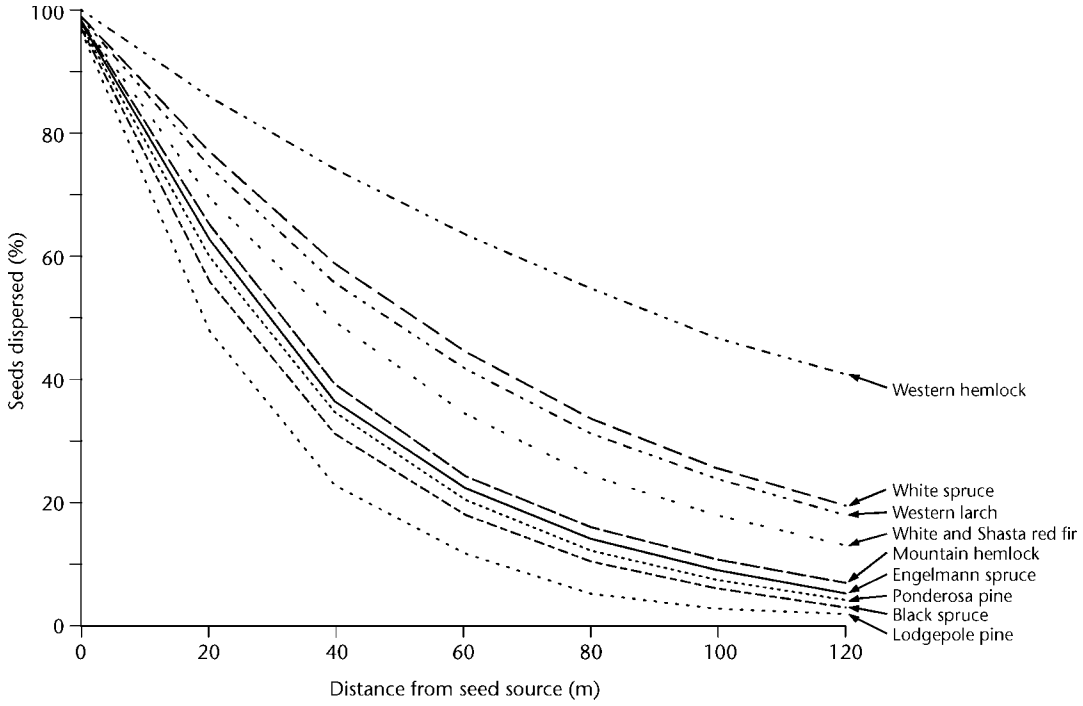


FIGURE 4.2 *Seed dispersal curves for nine conifers of the Inland Mountain West (McCaughey et al. 1986). Dispersal characteristics of different species can be compared by showing the relative number (percentage) of seeds found at different distances from the seed source.*

at least several kilometres from the nearest source (Zasada 1986).

Many external factors affect the distance that wind-dispersed seeds will travel. Wind speed is a primary factor, with strong winds dispersing seeds greater distances than light winds, and prevailing winds usually determining the direction of dispersal. Wind speed also affects abscission, but the speed required to cause seed abscission depends on how many abscission layers are present (Greene and Johnson 1989, 1994). The wind speeds required to actually achieve seed abscission in most species is demonstrably greater than median wind speeds reported for North American weather stations, resulting in seed dispersal distances being greater than would otherwise be expected (Greene and Johnson 1995). However, standard wind measurements are very different from wind speeds within the trees, which are usually lower; even high winds are rapidly reduced within the canopy. Thus, median wind speeds from a reference station can be used only as an index of general conditions.

Seeds are likely to travel farther from trees that are in exposed positions, such as on ridgetops. Within a stand, air speed will depend on stand density and structure. Stands where the trees are closely spaced will have low horizontal air speed.

Rising thermal winds can also disperse seeds uphill in mountainous terrain at mid- to lower elevations (McCaughey et al. 1986); updrafts are more important in dispersing light seeds. Dispersal may be more frequent in the early afternoon when more abscission events take place because of low relative humidity and high wind speeds (Greene and Johnson 1989).

Dispersal distance is also dependent upon the tree characteristics, size of the seed crop, and seed morphology (see Section 4.1.8). Seeds are likely to disperse farther from tall trees, from fruits or cones concentrated near the top of the tree, and from dominant trees.

There can be considerable variation within a single species in dispersal distance; such variation can affect sibling competition, density-dependent predation or parasitism, and the probability that seeds of particular individuals will locate canopy gaps (Greene and Johnson 1992). Intraspecific variation in dispersal distance may be due to differences in seed size and shape, but vertical and horizontal wind intensities are usually more important (Greene and Johnson 1992). Thus, the variation in dispersal distance between trees of the same species is probably due more to their location (and exposure to wind) than to genetic differences.

4.1.5 Quantity and quality of dispersed seeds

The regeneration potential of a site is often related to the quantity of filled seeds reaching the soil surface. This varies substantially among species and years. Many angiosperm species, such as *Betula* and *Alnus*, can produce substantially more seeds than conifers, and do so over shorter intervals (Eremko et al. 1989; Zasada et al. 1992; see also Tables 3.1 and 3.2). However, the germination of angiosperms such as *Betula* is generally lower than that of conifers (Zasada et al. 1992).

The quality of dispersed seeds may vary due to insect and disease attack (affecting cone opening and seed release), and the activity of animals, particularly birds. For white spruce, quality was highest when seeds were dispersed in September, but quality declined after that (Dobbs 1976; Zasada 1986). Other species such as paper birch disperse high-quality seeds both early and late in the cycle (Zasada et al. 1992).

4.1.6 Climatic conditions

Weather (e.g., rain, heat, storms, drought) can have a major influence on seed dispersal affecting both primary and secondary dispersal. Seeds may fall from the parent tree as the bracts and fruits dehydrate and dehisce (e.g., *Salix* spp. and most conifers), or the entire cone may disintegrate (e.g., *Abies* spp.). Abscission of seeds increases with higher lateral winds and lower humidity. Prolonged dry weather may cause cones to open prematurely, and storms may blow catkins and immature seeds to the ground.

Temperature also plays a major role in seed dispersal of several species. In British Columbia, lodgepole pine, black spruce, and jack pine have serotinous or semi-serotinous cones. Seeds are not released until the resin binding the cone scales is melted by fire or high temperature, so in years when temperatures do not reach a certain threshold, seeds may not be released. Winds are a major dispersal agent for seeds after a fire or hot weather, but logging can also be a dispersal agent when seeds are spread with logging slash (Fleming and Mossa 1996).

In species such as *Picea*, seeds are released while the cones are still on the tree, which allows the seeds to travel long distances. In *Abies* species, seeds are wind-disseminated after the cones disintegrate on the tree in September and October. Other conifer species release cones with the seeds still attached, so dispersal distances are relatively short for these species. Ponderosa pine cones, for example, fall to the ground with the seeds still inside. The seeds are released when high temperatures dry the cones and cause the scales to open.

The seeds of some species can germinate while still attached to the parental tree. Germination of maple seeds can occur on the tree after dormancy requirements have been met and if environmental conditions are suitable (Zasada 1991). The viability of these germinants depends on suitable conditions; low temperatures and low humidity can kill the seedlings before and after they are dispersed.

4.1.7 Dispersal patterns

The dispersal pattern of seeds is closely linked to the action of their primary dispersal agent. Regardless of dispersal mode, the highest levels of seedfall are found within the stand. Generally, the farther away from the source, the fewer the seeds, and the more variable the density. In animal dispersal, the pattern is more variable because dispersal is independent of wind direction.

Wind-dispersed seeds can be considered as coming from a point source (a single tree releasing seeds), or as an area source (a stand of trees dispersing seeds into an adjacent clearcut) (Greene and Johnson 1989). With wind-dispersed seeds, the highest numbers of seeds are found close to the source; the quantity of seeds decreases rapidly with distance downwind from the seed source, and continues at a low level (McCaughey et al. 1986). This results in a U-shaped distribution of seed dispersal across openings such as clearcuts (McCaughey et al. 1986).

In contrast, the distribution pattern of animal-dispersed seeds is highly variable. One example is the clumping pattern of whitebark pine trees resulting from seed dispersal by the Clark's nutcracker (Guiguet and Beebe 1973; Hutchins and Lanner 1982). Seeds distributed by frugivores follow a dispersal pattern similar to that of wind-dispersed seeds. Seedfall is highest near the source, but if the tail of the seed shadow crosses the edge of a treefall gap in the forest or bird perches in an open field, there can be another, smaller seedfall peak, which produces a bimodal dispersal pattern (McDonnell 1984; Hoppes 1988).

Secondary dispersal alters the primary dispersal pattern. The subsequent effects of wind or water on seeds on the ground results in the clustering of seeds within depressions. Rocks and vegetation can act as barriers to seed movement, catching seeds moving horizontally along the surface. Clustering can also result from animals moving seeds to caches.

4.1.8 Dynamics of seedfall

Specific morphological adaptations have evolved to aid the dispersal of wind-dispersed seeds. Some seeds have plumage, others have wings. Mechanically, plumage and wings operate in different manners (Niklas 1992); plumage causes parachute-like behaviour, wings cause helicopter- or glider-like behaviour (Table 4.1 and Figure 4.3). Such structures slow the rate of fall, allowing lateral winds to carry the seeds greater distances before coming to rest on the ground (Farmer 1997). The large plumage of *Populus* spp. acts as a parachute, decreasing the terminal velocity and allowing the seeds to remain airborne longer.

TABLE 4.1 *Seed dispersal mechanisms of winged seeds (after Niklas 1992; Farmer 1997)*

Wing type	Species examples	Mechanism
Plumage	*Populus*, *Salix*	Parachute: decreases terminal velocity
Winged		
Plane-winged	*Betula*, *Thuja plicata*, *Chamaecyparis nootkatensis*	Glider: linear gliding flight
Autogyroscopic	*Acer*, *Picea*, *Pinus*	Helicopter: autorotation creates lift

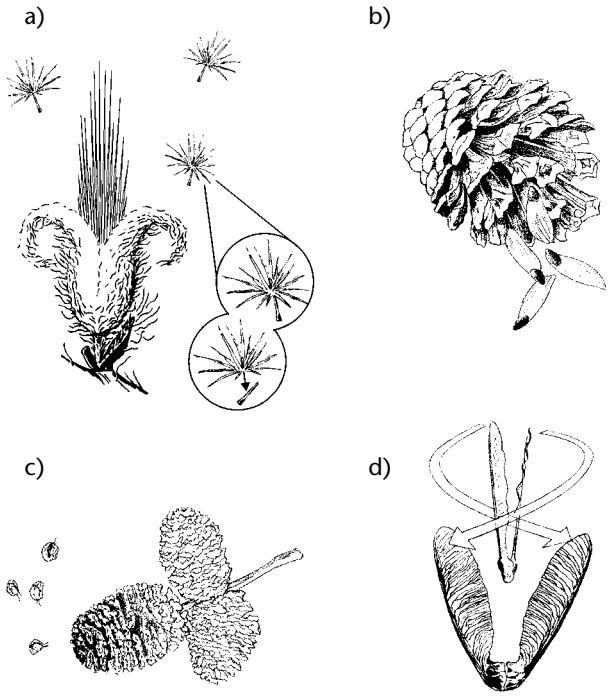


FIGURE 4.3 *Examples of dispersal mechanisms of winged seeds: (a) plumage (*Salix*); (b) winged-autogyroscopic (*Pinus*); (c) plane-winged (*Alnus*); (d) winged-autogyroscopic (*Acer*). Sources: (a) Zasada et al. [1998]; (b), (c), and (d) "*The Secret Life of Tree Seeds*" poster, B.C. Ministry of Forests, Victoria, B.C.*

Winged seeds or fruits are produced by gymnosperms (e.g., *Pinus*, *Picea*) and a host of angiosperms (e.g., *Acer*, *Betula*). There are two categories of winged seeds: plane-winged and autogyroscopic. In plane-winged seeds, the seed coat or fruit wall grows more or less symmetrically with the longitudinal axis, generating a more linear gliding flight. In autogyroscopic seeds, lift is created by wings that permit autorotation during free fall, such as the helicopter-like movement of maple samaras (Niklas 1992).

Plane-winged seeds and fruits benefit aerodynamically by concentrating their centres of mass in a position relative to the chord of their wings (see Niklas 1992), which stabilizes the location of the centre of pressure (where resultant aerodynamic forces act on the wing). The precise position of the centre of mass determines the incidence angle at which equilibrium occurs, this in turn determines the glide angle. The smaller the glide angle, the larger the dispersal range of the seed or fruit. For more information on the aerodynamics of plane-winged and autogyroscopic fruits and seeds, see Ward-Smith (1984).

The mean terminal velocity of seeds may vary considerably among populations of some species (e.g., lodgepole pine), while being fairly constant within other species (e.g., most spruces). The size of the seeds and of their wings determine the maximum speed of descent (terminal velocity) of a seed. Small seeds with large wings probably disperse farther than larger seeds with small wings (McCaughey et al. 1986). Variation in terminal velocity due to seed size could enhance dispersal distance (Greene and Johnson 1992).

The terminal velocity of seeds can be determined by dropping seeds in still air (e.g., within a closed stairwell) and measuring their descent time with a stopwatch. Reported values for representative western tree species are given in Table 4.2.

TABLE 4.2 *Mean terminal velocities reported for seeds (with seed wings attached) of some British Columbia tree species*

Species	Mean terminal velocity (m/s)	Source
Hardwoods		
Balsam poplar	0.28	Greene (unpublished)
Trembling aspen	0.39	Greene (unpublished)
Paper birch	0.55	Björkbom 1971; Greene and Johnson 1995
Conifers		
White spruce	0.57	Zasada and Lovig 1983
Western hemlock	0.60	Greene and Johnson 1995
Sitka spruce	0.60	Greene and Johnson 1995
Lodgepole pine	0.60–1.0	Greene 1990
Engelmann spruce	0.61	Greene and Johnson 1996
Black spruce	0.61	Greene and Johnson 1989
White spruce	0.62	Greene and Johnson 1995
Douglas-fir	0.93	Greene and Johnson 1995
Tamarack	0.96	Greene (unpublished)
Western redcedar	1.25	Greene and Johnson 1995

4.2. Approaches to Studying Seed Dispersal

Most basic studies of seed dispersal include documenting seedfall beneath a parental canopy. The most common approach to sampling seed rain is to use seed traps. Seed traps are suitable for seeds dispersed by wind as well as by vertebrates that ingest seeds or fruit and then defecate or regurgitate them. Pivotal to these studies is the determination of a suitable size, number, and placement of the seed traps. It is also important to keep in mind that it is viable seeds that are of interest in seed dispersal studies. See Section 7.2 for tests for seed viability.

Many studies use a one-dimensional approach, where seed traps are used to determine the seed dispersal distance in one direction (generally downwind). These types of studies produce a dispersal curve. A mathematical equation can then be fitted to the data. This approach is suitable when the objective is to determine the distances which tree seeds can disperse, or for making various kinds of comparisons. A one-dimensional approach can be used to study the genetic structure of populations, successional dynamics after natural disturbance, and tree invasion into grasslands, tundra, or old fields. In forestry, dispersal curves are used to evaluate prescriptions for natural regeneration in strip cuts, patch cuts, and clearcuts, which depend on a satisfactory density of tree seeds dispersing from the unlogged forest into the centre of the cutblock.

In a two-dimensional approach, data from seed traps are collected to provide information on the number and distance of seeds dispersed in all directions. A dispersal curve extending in all directions from a point source describes the seed shadow generated by an individual tree. A seed shadow is therefore an area of dispersed seeds, centred on (or downwind from) a seed-producing individual or stand. A two-dimensional approach would be used to determine the effectiveness of leaving seed trees in a clearcut to provide seeds for the natural regeneration of a site.

Seed rain variability and sampling problems make it difficult to accurately describe seed deposition in two dimensions. It is not always clear whether point-to-point differences in seed rain density are actually due to the change in distance from the source, or to sampling error. In conducting studies of seed rain, there are difficulties in replicating the sampling. Therefore, there is usually a high degree of uncertainty associated with descriptions of seed rain patterns (e.g., maps of seed rain density) within a stand or across a block.

To minimize the uncertainty inherent in documenting seed rain patterns over space, use the largest possible seed trap. With increasing seed trap size, there is a decrease in sampling error and the probability that trap-to-trap differences are due to chance. Although larger traps are more cumbersome, using 1.0 m^2 traps instead of 0.1 m^2 could reduce the uncertainty level by a factor of 10. If you are using small traps, cluster two to four seed traps around a point. Keep in mind that these are subsamples of one experimental unit; having additional traps does not increase the number of replications of the design.

An alternative two-dimensional approach may be taken when studying trees with large seeds (e.g., *Carya*, *Juglans*, *Malus*, *Quercus*, some *Acer* spp.) which fall primarily beneath their own tree crowns. Dispersal of large seeds can be effectively studied by mapping the resting location of all seeds, or determining seed density in sample quadrats within a fixed radius of the parental tree.

In a three-dimensional study, the seed shadow is followed over time. Most ecological studies of seed biology and plant demography are conducted over several years because of natural variation in seed production and other factors. The periodicity of natural seed production (Section 3.1) usually requires many years of monitoring to obtain a good picture of mean seed rain density and its variance.

Biologists have studied the fate of individual seeds, or even of artificial seeds (e.g., Augspurger and Franson 1987) under conditions of controlled release (e.g., Greene and Johnson 1989, 1995). Such studies are useful to assess the rate of fall and the horizontal distance travelled by different species when seeds are released from different heights or under different wind conditions. This information is typically used to describe the basic biology of a species (e.g., McCaughey and Schmidt 1987), or to subsequently model its dispersal behaviour (e.g., Greene and Johnson 1989).

It is possible to distinguish between primary and some forms of secondary dispersal on the basis of where the seeds are found (see Matlack 1989). For

wind-dispersed seeds, one could assume that most seeds found in elevated seed traps are the result of primary dispersal, and any seeds found underneath are the result of secondary dispersal (horizontal movement). For other types of secondary dispersal, seed density can be determined and then the area sampled again at a later date to determine if any of the seeds have been removed (see also Section 5). Secondary dispersal by water has generally been noted in the literature as an observation, because of the technical difficulties in tracing the movements of seeds.

4.3 Measurements and Methods

4.3.1 Basic considerations

Mapping the resting spots of large seeds can be facilitated by clearing the forest floor under the canopy of the parental tree and slightly outside the crown projection, or by spreading tarpaulins under the tree crown. Individual seeds can either be removed or marked (paint or nonphytotoxic compound) after counting to ensure they are not counted twice; this is especially important if repeated measurements are taken. Leaving marked seeds on the ground permits you to determine if they are being removed by birds or rodents. To study the timing of seed dispersal, seeds or fruits should be collected two or three times per day, which will provide a good estimate of the rate of dispersal (J. Zasada, pers. comm., 1996).

The design, distribution, and monitoring of traps are a primary concern of forestry researchers studying tree seed dispersal. Many studies of wind- and vertebrate-dispersed (ingested) seeds (see Hoppes 1988) measure seed dispersal by collecting seeds after they fall in some type of seed trap. You may find that collaborating with an ecologist interested in litter fall or entomology will maximize your sampling system, as plant detritus and insects are often caught in the seed traps.

The ideal height of the seed trap opening is flush with the forest floor, because it is the level at which seeds would naturally rest before germination. However, there are difficulties with low-lying seed traps: water may accumulate, seeds may be washed out, or rodents may enter the trap and eat the seeds. Seed traps may be set higher to avoid seed deflection by ground foliage, or to continue collecting seeds after snowfall.

A problem, especially for plumed seeds, is retaining them in the trap after falling. Many kinds of seeds can be lost through wind gusts, flooding, and movement by animals. Deep-walled seed traps, adhesive surfaces, cover screens, and frequent monitoring can help retain the seeds (see Section 4.3.2).

Some seeds are inevitably consumed before you can count them, and exclusion of predators may therefore be necessary. Most insects are so small it is difficult to exclude them, but mammals and birds can be excluded using coarse-weave wire screening or hardware cloth. Make sure the grid size is not too small to exclude any of the seeds of interest, nor large enough to permit rodents. An 8–10 mm grid size is good for most conifer seeds. (Refer to Table 3.6 for sizes and weights of British Columbia tree seeds; and Section 5 for more information about exclusion of predators). If it is important to know the portion that are secondarily dispersed by animals, it may be possible to modify some of the methods from Section 5 for this purpose.

The more frequently that you can monitor and count your seeds the better; detailed phenological interpretations are then possible (or may even be an objective of the study), and some of the risks discussed above can be minimized. Weekly monitoring is recommended during the period of active dispersal (unless more frequent monitoring is necessary); this can be decreased to biweekly or monthly monitoring the rest of the year. Some researchers count and evaluate seeds at the trap site; others prefer to bag the contents of each trap and evaluate them later. However, this last method requires organization and commitment to avoid a backlog of unexamined seeds.

A special problem is assessing the dispersal of seeds that can germinate on the tree, such as bigleaf maple (Zasada 1991) or true firs and western hemlock which can germinate either in cones in the tree or in cones on the ground, (e.g., squirrel caches). Since the dispersal of maple seedlings may occur many months after seed dispersal, seed traps would most likely be removed and sampling stopped before this event. It may be necessary to sample germination in the tree and in cones to determine their proportion of the total crop. While it might be useful to determine the success of these germinants in finding a suitable microsite after dispersal and surviving, that could

be very time consuming. By measuring the effective dispersal rather than seed rain, no distinction need be made between the dispersal of seed and germinants. Such measurements can be facilitated by deploying an array of trays filled with sterilized potting soil. Other methods are discussed in Section 7.3.

Generally it is difficult to measure dispersal by vertebrates that hoard seeds. Seed traps are not recommended as the sole measuring tool, but they can be used in combination with cache searches. Cache locations can be mapped, but with this method there is no way to determine what proportion of the seeds will remain uneaten, or their germination potential. Another method measures "effective dispersal," in which dispersal is inferred from the number of seedlings counted during the next growing season. To determine the amount of seed that is being removed by hoarding vertebrates, two sets of traps can be used: one that excludes predators and one that allows seed removal (see Section 5 for methods of measuring seed predation).

Measuring secondary dispersal over snow requires different techniques For example, the primary dispersal agent for *Betula lenta* is wind, and seed release is from September through March. Secondary dispersal occurs when seeds are moved horizontally across the snow. Both primary and secondary dispersal can be monitored simultaneously if the seed trap for primary dispersal is suspended above the snow: any seeds that accumulate under the trap must have arrived there by horizontal movement. Snow under the trap (the top 2 cm) can be collected from traps or protecting platforms, and melted to extract seeds for counting (Matlack 1989). Total seed deposition by both primary and secondary means can be estimated by collecting snow samples from unprotected areas. To limit the impact of footprints in the snow (because seeds accumulate in hollows), treatment plots should be approached from downwind. It is also possible to control for seeds present in the snow before the beginning of an experiment by covering additional quadrats with polyethylene sheeting to prevent any seed deposition during the experiment and then sampling them at the end (Matlack 1989).

Secondary dispersal in water by rivers is important to species that live on floodplains. Seeds are also moved from their original positions by rain. Reports of these phenomena appear in the literature as observations, and no methods have been found for measuring the quantity and distance of this type of dispersal. Potential methods include mapping the presence of seeds in "deposition" sites such as river bends compared to other locations; using nets or other barriers to collect seeds; and analyzing isozymes of individual trees or clones (since poplars and other floodplain species tend to spread by coppicing) and comparing "downstream" and the "upstream" individuals.

4.3.2 Seed trap design

The most widely used seed trap is a fixed-area tray, usually rectangular, which excludes rodents with 8 or 10 mm grid hardware cloth. Adjustments will have to be made for some larger seeds, such as oak and bigleaf maple, as the 8–10 mm grid size is too small for them to pass through. The same trap design could be used for both wind- and vertebrate-dispersed seeds. For more information on designing traps for predator exclusion, see Section 5.

Proper drainage must be provided, as well as an easy way to remove and count seeds. Large aluminum baking pans and plastic greenhouse trays have been used as the basic seed trap, with holes punched for drainage and hardware cloth crimped over the top to keep out birds and rodents. A good basic design is one with a wooden frame, with metal window screening (about 1 mm grid) stapled to the bottom, allowing water drainage but retaining seeds (McCaughey and Schmidt 1987; Youngblood and Max 1992). A wooden lid with permanently stapled hardware cloth (Figure 4.4a) eliminates the need to repeatedly unstaple the hardware cloth. The lid must be precisely crafted to fit tightly over the base with no room for rodents to enter. Other trap designs are shown in Figure 4.4. A seed trap with a wooden lid and permanently stapled hardware cloth (Figure 4.4a) will deter predators and provides easy access for monitoring. A trap made from a galvanized flue thimble (Figure 4.4b) can be placed flush with the surface, but leaves accumulating on the surface may blow away resulting in the loss of seeds. A trap made from a tractor funnel (Figure 4.4c) will retain leaves and seeds. The recessed surface will help to retain the contents.

Species with light seeds and those with appendages for air flotation (like those of dandelion, fireweed, aspen, and willow) require specialized traps. Other traps do not work as well for these species because the seeds can easily be wafted back out of the trap. Sticky traps consisting of an adhesive such as Tanglefoot™ on replaceable index cards, filter-paper disks, or cardboard sheets have been found effective. Sticky traps are messy to make and maintain, and they capture detritus and insects as well as plant seeds, thus should only be used when absolutely necessary. In areas where wind and dust are prevalent and traps for light seeds are required, funnel traps and glued-soil depression traps work well (Johnson and West 1988). For light seeds such as *Betula* and *Alnus*, a deep-walled funnel trap with walls above the rodent-excluding screen can prevent seeds from wafting out of the trap (Zasada and Gregory 1972; Figure 4.4c).

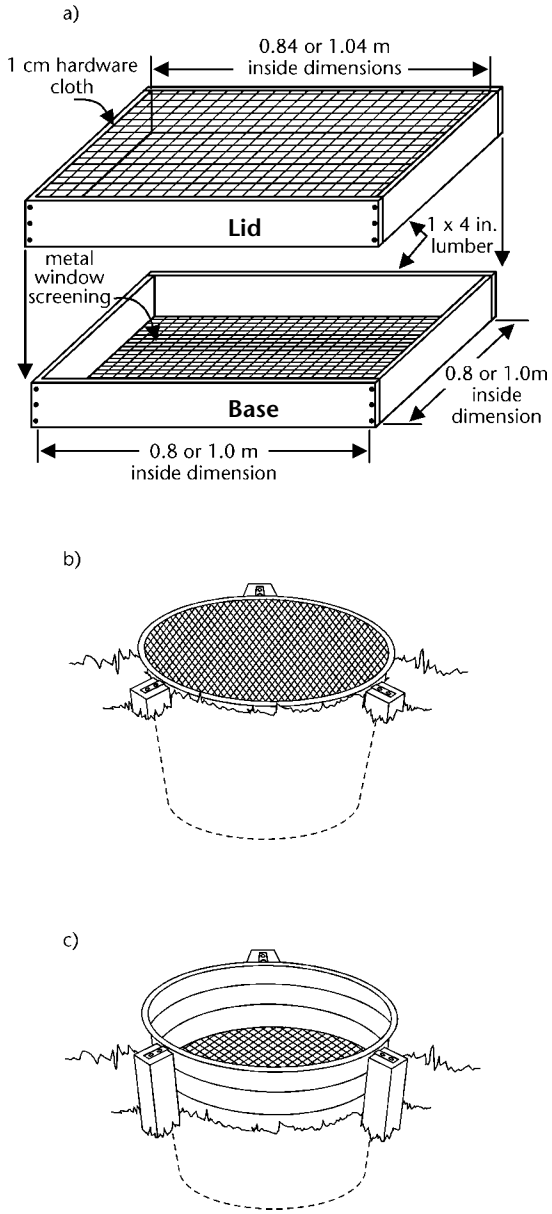


FIGURE 4.4 *Seed trap designs: (a) a trap with a wooden lid and permanently stapled hardware cloth; (b) a trap made from a galvanized flue thimble; (c) a trap made from a tractor funnel. Source: (b) and (c) after Zasada and Gregory (1972).*

Water-filled trays have been used to capture and retain willow and aspen seeds (Walker et al. 1986). The dispersal unit of these seeds usually stays attached to the water surface once it lands and, because these species germinate relatively quickly in water, viability can be tested at the same time (Wyckoff and Zasada [1998]). Wet soil is also a good medium for catching and holding these types of seeds (Zasada et al. [1998]). To avoid freezing when measuring seed dispersal during winter, kerosene can be used rather than water (Matlack 1989). Both water- and kerosene-filled trays should be inspected frequently (even daily) because leaf litter can accumulate and decrease the effective seed collection area.

To exclude rodents, a bag or tray can be suspended above the forest floor (Hughes et al. 1987), and the guy wires or a flexible tripod of intersecting wires can be used to hold open the mouth of the bag. However, rodents can still climb into the traps with the aid of vegetation or snowpack. Cattle, bears, or heavy snow may also cause traps to collapse. To facilitate the handling of suspended bags, Velcro™ tabs are recommended to attach the bags to the frame.

A combination design has flexible, replaceable bags (e.g., nylon window screening, woven grain bags, polyester cheesecloth, or remay) that can be changed regularly and the contents tallied in the laboratory. The bags are held within a rigid frame (similar to Figure 4.4a without the bottom screen) which is laid on the forest floor and covered with a rodent-exclusion screen.

Another approach is to fill traps with potting soil, and then count the number of seeds that germinate.

4.4 Experimental and Sampling Design

4.4.1 Estimating seed rain density

Replication is a major problem in many studies. Both replication of seed traps within a treatment site and replication of treatment sites can be limited by time and money. For example, to compare seed rain density in a treatment and a control site, a researcher must decide how many samples (seed traps) will be taken, and how many treatment and control sites to use. A common design in silviculture is the paired-site experiment. In this design two adjacent and presumably similar sites—a treatment site (e.g., clear-cut) and a control site—are compared, then the difference between the two is monitored. Often several samples are taken from each site. The assumption is that any observed difference after treatment is due to the treatment. In reality, the lack of replication of the experimental units (sites) means that you cannot separate the site effect from the treatment effect in the predicted error value. For a more complete discussion of experimental design, see Sections 1.4 and 3.6 and MacDonald and Stednick (1994).

Determining the number of seed traps needed within a site should be done very early in designing your experiment in consultation with a statistician. A pilot study or data sets from similar experiments may be used to make a preliminary assessment of the seed rain variability and determine the number of seed traps required to capture that variability. Time and money restrictions may preclude installation of all the traps required. Compromises are made in most studies, and it may be preferable to limit the number of seed traps rather than limit the number of experimental units (sites). In general, it is most efficient to use nested sampling to characterize your variability, instead of taking one large sample. For example, by taking four smaller subsamples from one site, variability can be estimated within the site. However, remember that these are subsamples, not replicates; to treat them as replicates would be pseudo-replication (Hurlburt 1984; MacDonald and Stednick 1994).

The number of seed traps should increase with increasing distance from the seed source (Hoppes 1988) because seed rain density decreases and variability increases with distance from the seed source. As a general rule, the number of seed traps should double for each tree-height away from the seed source. Although the number of traps can be fewer nearer the source, it may be problematic if near traps are clumped together (D.F. Greene, pers. comm., 1996). Unless the source is a monoculture, the non-random distribution of species requires that traps be spread out along the forest edge. Indeed, because of this non-random distribution of source trees, you should avoid having a small number of traps near the forest boundaries.

Increasing the sampling intensity with distance is especially important when identifying the maximum dispersal distance (Portnoy and Willson 1993). The final distance will depend on such factors as the size, weight, and aerodynamics of the seeds. For example, sampling dispersal of seeds that can travel long distances, such as willow, will require a larger number of traps laid out over a larger area than a species such as subalpine fir, which has a shorter dispersal distance. The strength and direction of the prevailing winds will also affect the placement of seed traps. In particular, consider the frequency of gusts which may cause abscission of the seed, and updrafts which may result in seeds travelling beyond the limit of the study layout.

The problem of how much total trap area is required at each distance will depend most strongly on source density and, sadly, luck. Luck is involved because many species are markedly variable in the production of seeds. Most have temporal coefficients of variation of 0.8 (oaks, pines) up to almost 2.0 (most conifers, a few hardwood species). Years of virtually no seed production occupy about 30% of long-term records. A simulation performed on a 33-year record from New Zealand showed that sampling for 7 consecutive years would be required to obtain apparent mean production values within two-fold of the observed long-term mean (D.F. Greene, pers. comm., 1996). Ideally, therefore, an investigator must either wait to put in the traps until binocular estimates indicate a reasonable seed supply, or be willing to continue the study for several years. However, you can estimate the total trap area required.

Using data from long-term studies, Greene and Johnson (1995) estimate mean annual seed production per square metre (Q) as:

$$Q = 3067\ \bar{B}^{0.92}\ m^{-0.58}\ N\ ,$$

where: $\bar{B}$ = mean source basal area (m^2),
m = individual seed mass (grams), and
N = source density (number/m^2).

For example, suppose you wish to estimate seed production in a stand of Engelmann spruce (mean seed mass 0.003 g, mean basal area of source trees 0.01 m^2, and density of source trees 0.1/m^2). Well inside the forest in an average year you could expect about 130 seeds/m^2, and at the forest edge, about half that amount (65 seeds/m^2). As a rule of thumb, at 100 m from the forest edge you could expect about 4% of the deposit well within the forest (D.F. Greene, pers. comm., 1996), or about 6.5 seeds/m^2. Thus, to register a single seed at 100 m, quite a large trap area is required. To be cautious, the minimal estimate of the required trap area should be increased 10-fold, because you may well be sampling in a poor year. In general, investigators use relatively small amounts of trap area, and published studies may therefore be dominated by "mast-year" examples (D.F. Greene, pers. comm., 1996).

You may wish to examine the final resting spots of individual seeds, in which case the horizontal distance and direction travelled may be noted (e.g., Zasada et al. 1992), or the microsite attributes of resting spots recorded (e.g., Janzen et al. 1976).

Whatever the measurement method employed, each seed trap should provide a single value for the number of viable seeds intercepted per square metre per year. This point-measurement of seed rain density is usually the standard unit for further analysis. The point measurement is often extrapolated from seed traps with collection areas of less than 1.0 m^2, or is averaged over several years. Usually only filled, viable seeds, or the ratio of viable seeds to the total captured, are of interest. See Section 7.2 for methods to determine viability.

Since many study areas do not have analogous treatments replicated in separate stands, it is often more practical to replicate seed rain studies over time, (e.g., by measuring treatment and control sites in three consecutive years). Several precautions should be taken: take samples at essentially the same time from the treatment and control sites; ensure that the control site is comparable to the treatment site; and check that local events that affect trends do not occur at one site but not at the other (MacDonald and Stednick 1994).

When to start and stop, and how often to sample will be determined by the timing of seed release of the species. It is best to sample more frequently during the period of heaviest seed rain, usually the first several weeks of seed release. Weather conditions may also influence your schedule; sampling should be more frequent in windy conditions, since wind increases abscission rates, and seeds may be removed from traps by wind and rain.

Site information should be collected to characterize the study area (see Section 1.6). However, if you are modelling how seeds disperse from a tree or from a stand edge, some additional data will be required:

- seed rain density under the tree, or within the intact stand;
- approximate heights from which the seeds are released;
- seed samples to measure mass and area to determine wing loading, and terminal velocity when dropped in still air (Greene and Johnson 1989);
- information on dispersal agents, such as prevailing wind velocities at different heights (Section 2.3.4) and relative abundance of seed-dispersing vertebrates;
- landscape features that impede (or enhance) the flow of wind or water, or attract or repel seed-carrying birds or mammals (Johnson et al. 1981).

Estimating seed rain from a point source

To study seed dispersal from a single source (individual tree), for both wind- and vertebrate-dispersed seeds, the source tree should be sufficiently isolated from other trees. Contamination of seed traps with seeds from nearby trees is probably the most significant problem in this type of study. The type and size of trap used will depend on the seed

characteristics of the species being studied and the types of predators expected (see Section 4.3.2).

The layout of traps around a point source is the same for both one-dimensional and two-dimensional studies, except that sampling may be more intensive in a two-dimensional study. The objective of a one-dimensional study is to derive a dispersal curve (as described in Section 4.2) in one dimension; the objective of a two-dimensional study is to map changes in seed rain in two dimensions. The difference between one- and two-dimensional studies lies more in presentation of the data than in the design. For both types of studies an array of traps is arranged around the parental tree. While the ideal trap arrangement should be random, it does not account for the variability of seed dispersal increasing with distance from the source (thus the number of seed traps should also increase with distance). For this reason most seed-trap layouts use a systematic design—a circular arrangement with the diameter of the circle increasing with distance from the starting point (Figure 4.5), or a systematic linear arrangement (Figure 4.6).

When species produce large seeds dispersed by gravity, the seeds primarily fall directly beneath the parental trees. Three methods are available: each seed location can be mapped, seed traps may be set out, or density determined in an array of survey quadrats (see Sections 4.2 and 4.3).

Estimating seed rain from an area source

To estimate the overall seed input to a unit of land with seed-bearing trees, seed traps should be distributed randomly throughout the stand or treatment area. Such layout patterns are appropriate for mature uncut forest stands, and under uniform shelterwood and seed tree canopies. When studying a stand composed of a single species, or if you are only interested in the seed dispersal characteristics of one species, a trap suitable to just that species should be chosen.

For seed rain mapping, sampling on a regular grid is more practical than random sampling (for examples, see Carkin et al. 1978; Noble and Ronco 1978; Alexander 1986; McCaughey and Schmidt 1987; Matlack 1992; Youngblood and Max 1992). If a grid is used, ensure that the grid interval is not a multiple of mean inter-tree distance or mean crown diameter. For example, if the trees are very uniform and have a mean crown diameter of 5 m, grid sampling at 5, 10, 15, or 20 m (etc.) would have a greater-than-random chance of repeatedly sampling under the same part of the trees (e.g., adjacent to the bole, or at the periphery of a tree crown).

Seed-trap layouts suitable for quantifying the seed input into a forest opening would use a systematic layout (two variants are shown in Figure 4.6). The number of seed traps increases as distance from the area source increases, permitting a researcher to sample rare long-distance seedfall events. Although such sample arrays are two-dimensional, they describe seed dispersal in only one dimension, with multiple transects of seed traps serving primarily as subsamples for describing seed input at different distances from the seed source. Results are portrayed as seed dispersal curves, as shown in Figure 4.2.

Comparisons between tree species are generally based on stand-level measurements. To study species

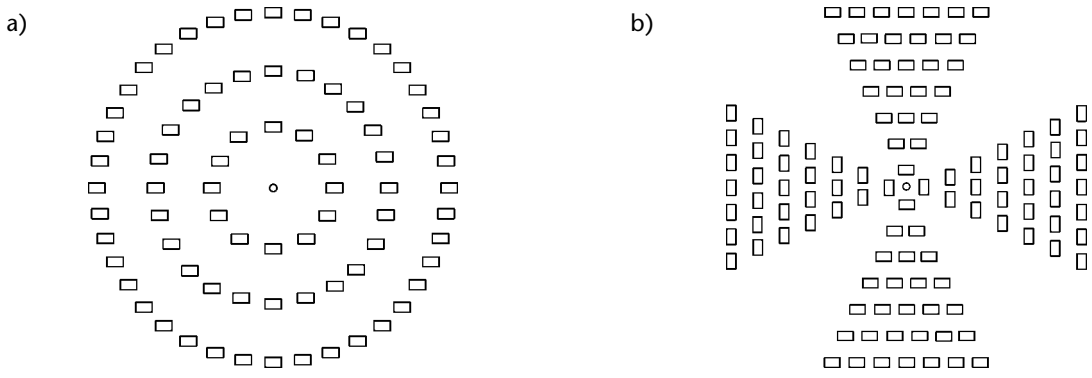


FIGURE 4.5 *Schematic of the distribution of seed-traps placed around a point-source. Each box represents a seed trap. (a) concentric circles; and (b) a cross (after Hoppes 1988).*

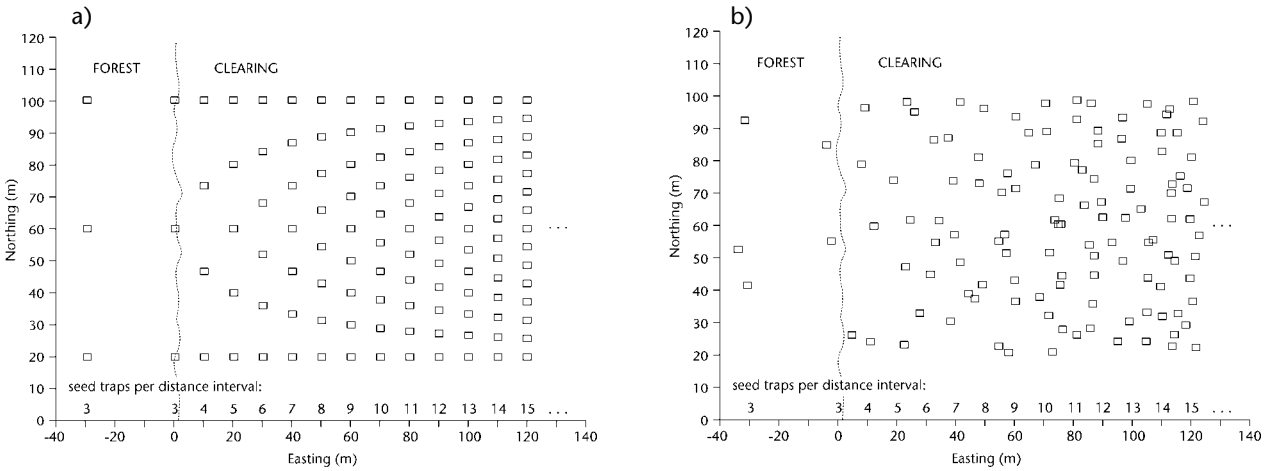


FIGURE 4.6 *Recommended seed-trap layouts at a forest edge for an area source. Sampling density increases with distance from the seed source: (a) traps distributed at regular 10 m intervals; (b) traps distributed randomly within each distance category.*

at the stand level, several seed traps (suitable for the species under study) must be used in each stand to provide a reliable measure of seed rain density. The number of traps within a stand has a relatively small effect on the overall power of a test, so it is better to have more stands per treatment than to have more traps per stand. When seed production levels are low, seed rain becomes more variable and spotty, so the number of seed traps per stand should increase to sufficiently characterize the variability. It usually requires many years of monitoring to get an accurate picture of mean seed rain density. The number and distribution of traps should be based on what is required to adequately sample in the poorest seed year.

The most difficult situation occurs if the composite seed rain of an entire tree community is to be sampled. The type(s) of trap used will depend on the different species being sampled. For example, to document seed rain of all trees into a boreal forest site might mean collection of the wind-dispersed seeds of birch, aspen, balsam poplar, willows, white and black spruce, balsam fir, and lodgepole pine. Questions a researcher must ask are: What kind of trap arrangement and what type of traps would be needed (spatial and temporal)? Could the same type of trap be used for all species? Would the trap array have to differ among species? Would trapping "season" differ? An array of different kinds and densities of seed traps, monitored at different times, will probably be required to obtain a good picture of overall seed inputs. Long-term studies (5–10 years or more) may be necessary to get a reasonable idea of annual periodicity of seed rain (D.F. Greene, pers. comm. 1997).

4.5 Data Analysis

The analysis of seed rain data follows the norms of most sample surveys and of those experiments that depend upon sampling within experimental units.

If you are also interested in spatial patterns of seed dispersal, then specialized statistical tools may be required. So far, modelling of seed rain patterns is really only feasible in the simplest scenarios (e.g., around widely spaced seed trees or at cutblock edges).

4.5.1 Descriptive analysis

Single-source and area-source (stand-level) seed rain density can be described by a measure of the central tendency (e.g., mean, mode, or median values) and the variation (e.g., standard deviation or 95% confidence interval) in trap-to-trap measurements. Seed traps are often deployed to determine what proportion of the forest area under study receives an adequate seed rain density (i.e., density above some arbitrary threshold) and an even distribution (dispersion) of viable seeds (i.e., high densities in some traps may not compensate for few or no seeds in other traps). In stands where extreme values prevail, using

the mode or median to describe central tendency is usually more appropriate than using the mean. This would be the case, for example, where there are mostly zero or many seeds per tray, rather than an even (or constant) range in the number of seeds per tray. In these situations, the spread about the norm is better described using percentiles or quartiles rather than standard deviation or confidence intervals. Results (in significant digits) should be expressed in terms of the method or measurement employed (size of seed trap and sample size, or total seed trap collection area, in this case).

4.5.2 Comparative analysis

Treatment comparisons can follow standard analysis of variance (ANOVA) approaches. This assures that the variance is appropriately partitioned. For example, the comparison of seed rain under four different silvicultural treatments in three separate stands might be as follows: all of the four treatments, T, would have to be applied within each stand (e.g., number of blocks = three), with extensive subsampling in each treatment unit (assume $p = 35$ subsamples), and over several years (assume $y = 5$ years). The ANOVA table and appropriate F-tests would then be set up as in Table 4.3. If the number of seed traps differs among stands, observations should be weighted by the number of subsamples per treatment cell when conducting ANOVA or regression.

When the number of subsamples in the treatment cells varies, the degrees of freedom (df) also vary: if individual counts are P_{ijk},

then:

$$\text{total df} = \left(\sum_{i=1}^{t}\sum_{j=1}^{n}\sum_{k=1}^{y} p_{ijk}\right)^{-1} \text{, and}$$

$$\text{subsample df} = \sum_{i=1}^{t}\sum_{j=1}^{n}\sum_{k=1}^{y} (p_{ijk} - 1)$$

where:

i = treatment,
j = stands, and
k = years.

Multiyear data collected from the same fixed network of seed traps can be treated as a split-plot-in-time ANOVA, as laid out in Table 4.3. If data are collected over time from the same sampling units, and the data from year-to-year are dependent, then a repeated-measures analysis would be appropriate. The main objectives of repeated measures analysis are to check if there is a trend over time, to take into account the inherent variation of a site, and to see if the trend is the same for all treatment levels. Repeated-measures data can also be analyzed using multivariate ANOVA with the multiyear data as response vectors. Refer to Moser et al. (1990), Potvin et al. (1990), Meredith and Stehman (1991), Gumpertz and Brownie (1993), and Nemec (1996) for more detailed discussions and a wider range of options for the analysis of repeated measures (see also Section 1.4.1).

If significant effects are found, a suitable multiple comparison method may be used to identify which means are significant. However, if your goal is to determine if a treatment—or several treatments—were significantly different, sufficient information may be gained by the ANOVA. Presentation of your ANOVA results, with a graph of your data, generally provides an effective visual comparison between treatments. It may not be necessary, or even relevant, to have a comparison between plot A in year 1 and plot D in year 3. If you do require multiple comparisons, Day and Quinn (1989) suggest Scheffé's method for

TABLE 4.3 *Split-plot-in-time analysis of variance (ANOVA) table for a hierarchical sampling design*

Source of variation	Degrees of freedom	Test for effect
Stand, N	$(n\text{-}1) = 2$	—
Treatment, T	$(t\text{-}1) = 3$	MS_T / MS_{N*T}
$N*T$	$(n\text{-}1)(t\text{-}1) = 6$	—
Year, Y	$(y\text{-}1) = 4$	MS_Y / MS_{Y*N}
$Y*N$	$(y\text{-}1)(n\text{-}1) = 8$	—
$Y*T$	$(y\text{-}1)(t\text{-}1) = 12$	MS_{Y*T} / MS_{Y*N*T}
$Y*N*T$	$(y\text{-}1)(n\text{-}1)(t\text{-}1) = 24$	—
Subsamples, $P(TNY)$ (subsamples nested within treatments, stands, and years)	$(p\text{-}1)tny = 2040$	—
Total:	$tnyp$ - 1 = 2099	

Note: Lowercase letters represent the number of levels of the sources, (e.g., Treatment (T) has four levels, t = four treatments; n = three stands; p = 35 subsamples; y = 5 years).

unplanned comparisons, or Dunnett's test when a control is compared to all other treatments. A small number of planned contrasts (not more than the degrees of freedom for that source) do not need any multiple range type of correction, the *p*-values of their associated *t*-values or *F*-values can be used without correction. For many non-orthogonal contrasts, a Bonferroni correction can be used; otherwise use Scheffé's method, since it is naturally very conservative. For more information see Milliken and Johnson (1992). Duncan's multiple range test and the Student-Newman-Keuls (SNK) test are also commonly used. Refer to Sit (1995) for more discussions on ANOVA and multiple comparisons for various types of experimental designs.

4.5.3 Regression analysis

The main objective of many seed dispersal studies is to relate seed rain density to distance from the seed source. The relationship of seed rain density, or the probable fate of any individual seed, is well described by the dispersal curve. The typical shape of the frequency distribution of seeds as a function of the distance from the source plant is a concave negatively sloping line (Zasada 1986). The two common mathematical expressions for the dispersal curve are: the inverse power model (Gregory 1968):

$$y = ax^{-b},$$

where: y = the probability density associated with the dispersal,
x = the distance from the source, and
a and b = unknown values that can be empirically derived;

and, the negative exponential model (Frampton et al. 1942):

$$y = ae^{-bx},$$

where: y = the probability density associated with the dispersal,
x = the distance from the source,
e = the base of natural logarithm (2.7182818), and
a and b = unknown values that can be empirically derived.

This equation was used by Youngblood and Max (1992) to describe seeds originating within a mature forest and dispersing from a forest edge into a clearing.

Advanced statistical regression analysis of seed dispersal data typically consists of (iterative) nonlinear curve fitting; see Sit and Poulin-Costello (1994) for details on fitting data to the negative exponential model and related functions using the SAS statistical package. There are advantages and disadvantages to both these models. The inverse power model has the advantage that it transforms to a straight line when it is plotted on log-log paper. Thus the numerical values of a and b can easily be estimated. The negative exponential model transforms to a linear relation when plotted on semilog paper, and the probability density y remains a finite number as the distance from the source x converges to zero. The disadvantage of both models is that neither provides any insight into the mechanistic attributes of dispersal and their effects on dispersal curves.

There appears to be little difference between the inverse power and negative exponential model in predicting actual dispersal curves (Niklas 1992). Both models have difficulties in predicting dispersal curves because variations in weight, size, and shape of seeds or fruits will affect the dispersal curve. The height at which seeds are borne on the plant, the ambient wind speed, and directional components (lateral wind direction) causing seed release or abscission will also affect dispersal curve characteristics. There is also the problem with describing the number of seeds plotted as a function of distance from a point source. The inverse power and the negative exponential models adequately describe one-dimensional dispersal curves, but in reality seeds are seldom disseminated in this manner; spatial distributions in two dimensions are more common.

With more complex statistical models, it is possible to incorporate additional information such as the "strength" of the seed source. For example, the basal area of trees at the seed source has been incorporated as an additional independent variable to predict the density of Engelmann spruce seeds at various distances downwind of a seed source (McCaughey and Schmidt 1987). Other terms could be added to provide a more accurate picture of seed dispersal, such as release height above the ground, terminal velocity of the seeds, and wind velocity.

4.5.4 Spatial analysis

Seed dispersal in two dimensions can be described by generating a map of seed rain density. Data are collected from a systematic array of seed traps, and interpolated between observation points to generate an isopleth map of seed rain density (e.g., Engle 1960; Augspurger and Hogan 1983). This can be done with standard interpolation algorithms in statistical packages such as SAS and SYSTAT, with or without various smoothing options. The problem with this method is the assumption that no sampling error occurred (i.e., that all trap-to-trap differences represent real trends in seed rain density over the distances concerned). These types of errors can be reduced by the use of large traps and replication over time.

Refer to Robertson (1987), Isaaks and Srivastava (1989), Legendre and Fortin (1989), and Rossi et al. (1992) and their references for more details on the use of geostatistics. These techniques are presumably applicable to describing trends in seed predation (Section 5) and seed bank patterns (Section 6) as well. Some commercial software packages for geostatistical analysis are listed in Appendix C.

4.5.5 Mechanistic modelling

In addition to statistical methods, mechanistic models are another option for fitting observed trends in seed dispersal behaviour. These models predict median horizontal distance travelled by the seed crop away from a single tree, using information such as the release height above the ground, horizontal wind velocity, and the terminal velocity of seeds falling in still air. For example, Okubo and Levin (1989) applied a tilted Gaussian plume model to predict the mode of the dispersal curve:

$x_m = h\, U_a / U_s$ for heavy seeds or fruits ($U_s > 1\ ms^{-1}$)

and

$x_m = h\, U_a / W^{\star}$ for light seeds or fruits ($U_s < 1\ ms^{-1}$)

where x_m = the modal distance,
h = the height of seed release,
U_a = the ambient wind speed,
U_s = the seed terminal velocity, and
$W^{\star}$ = the vertical airflow mixing velocity attending turbulence.

For information on other mechanistic models, refer to Greene and Johnson (1989, 1995, 1996) and Andersen (1991).

An advantage to the mechanistic approach is that data from a variety of plants growing in very different habitats can be placed within a single objective classification scheme based upon the aerodynamic properties of airborne spores, pollen, seeds, and fruits. However, most models make a large number of assumptions (Greene and Johnson 1995), so the resulting predictions may be far from reality, in particular for light seeds. Seeds of willows and poplars are often carried above the height of formation (where they are produced). Most mechanistic models do not address the question of how long seeds stay airborne and how that relates to the distance travelled. In a study on white spruce (Zasada and Lovig 1983), seeds were found to follow very complex flight patterns that may or may not be based on terminal velocity. Direct observation reveals a substantial number of ups and downs in the flight path of individual seeds. *Populus* and *Salix* seeds also follow complex flight paths that would seem to defy description by a mechanical model (J. Zasada, pers. comm., 1996).

SECTION 5 SEED PREDATION

Every part of nature teaches that the passing away of one life is the making room for another.
(Henry David Thoreau)

5.1 Background

Studies of seed predation are important to understanding how seeds are lost between dispersal and germination. Research has shown that seed predators can account for over 50% of the loss of a viable tree seed crop (Gashwiler 1970), including between 30 and 80% of the cones removed by squirrels (Hurly et al. 1987). In severe cases, up to 95% of the seed crop may be destroyed by predators, which can exert a significant influence on patterns of plant recruitment, plant species diversity, and plant community structure. Not only can seed predation affect the process of natural regeneration but it can also undermine the success of artificial seeding efforts.

A further rationale for studying seed predation is the lack of knowledge about the nature and magnitude of seed predation on tree seeds of western North America, especially hardwood species. While predation of conifer seeds was investigated fairly extensively from about 1930 to 1960, research has tapered off in recent decades. This loss of interest may coincide with the rise of reforestation by tree planting rather than by natural regeneration or aerial seeding. The current strong interest in maintaining "natural" levels of biological diversity (e.g., tree genetic diversity) as well as the search for more cost-effective means of reforestation may help to renew interest in natural regeneration. Unfortunately, there is a lack of recent published accounts describing or assessing methods used to carry out seed predation investigations. The material that is available is often unclear or incomplete; this section seeks to address the information gap by describing and assessing some common methods for studying seed predation. This section deals with predation on seeds that are mature; predation on seeds that are not fully developed is discussed in Section 3.5.2.

Seed predation studies usually have the objective of determining the number of seeds lost to predation. Questions that might be asked in seed predation studies include:

- Which species or species groups are preying on the seeds?
- Which species of seeds are being preyed upon?
- What proportion of seed loss can be attributed to predation versus other losses?
- What proportion of seeds are lost to predation by specific predators/predator groups?
- What proportion of seeds are lost to predation before versus after dispersal?
- How does the pattern of predation change over time in the short term (single year) and the long term (multiyear)?
- How does predation differ among different disturbances, ecosystems, sites, or other factors?
- How does providing alternative foods and seed mixes affect seed predation?
- How does seed predation affect recruitment of new trees?
- How do fluctuations in predator and prey populations affect seed predation?

5.2 Seed Predators

Although studies of the predators themselves are an essential component of seed predation research, describing detailed methods for taking a census of seed

predators is beyond the scope of this manual. Instead we provide basic information about common seed predators, suggest when and why we should study them, and include references for detailed methodology.

There are three main groups of seed predators: small mammals, birds, and invertebrates. Of these, seed predation by small mammals has been the most studied (West 1992). Although the role of birds and invertebrates in seed predation is less well documented, these groups can also destroy significant amounts of seeds (Gashwiler 1970; West 1992). Within all three groups, some species specialize in feeding in trees while others are ground feeders.

Mice, voles, shrews, chipmunks, and squirrels are small mammals that commonly eat tree seeds. In British Columbia, the deer mouse (*Peromyscus maniculatus*) is perhaps the single most important consumer of tree seeds. Chipmunks (*Eutamias townsendii* on the coast and *E. amoenus* in the interior) and voles (*Microtus* and *Clethrionomys* species in the interior) have also been identified as significant seed predators (Sullivan et al. 1990). Red squirrels (*Tamiasciurus hudsonicus*) are known to harvest 30–80% of the cone crop of some conifer species, and up to 96% of the cones from any one tree (Hurly et al. 1987; West 1989).

Bird species that prey on seeds or cones before dispersal include hairy woodpeckers (*Picoides villosus*), Clark's nutcrackers (*Nucifraga columbiana*), red crossbills (*Loxia curvirostra*), and white-winged crossbills (*L. leucoptera*) (Eremko et al. 1989). About 88% of the annual diet of red polls (*Carduelis flammea*) consists of paper birch and *Alnus* spp. seeds (White and West 1977). Common ground-feeding species include juncos (*Junco oreganus*), varied thrushes (*Ixoreus naevius*), song sparrows (*Melospiza melodia*), fox sparrows (*Passerella iliaca*), white-crowned sparrows (*Zonotrichia leucophrys*), pine siskins (*Carduelis pinus*), and golden-crowned sparrows (*Zonotrichia coronata*) (Eremko et al. 1989).

Insects are the most commonly studied group of invertebrate seed predators, although some attention has also been paid to mollusks. All conifers have insect species complexes that attack their reproductive structures (Finck et al. 1990). Some examples of insect seed predators are the western conifer seed bug (*Leptoglossus occidentalis*), which attacks the seeds of various species from British Columbia to Mexico, and the ponderosa pine seed moth (*Cydia piperana*), which is especially destructive to pine seeds in western North America. For more information on cone and seed insects, see Hedlin et al. (1980); common insect pests of conifer cone and seed orchards Table 21.1 in Finck et al. (1990). For information on diseases, which are another form of seed predation, see Sutherland et al. (1987).

For most research, knowing what species or species groups are eating seeds will be enough, although it is always helpful to know which are most abundant. Wildlife and insect pest inventories can be valuable sources of information on the species of small mammals, birds, or invertebrates present in the study area. For simple observational techniques, see Bookhout (editor, 1994) for small mammals and birds, and Southwood (1978) and Pedigo and Buntin (1994) for invertebrates.

Seed predator censuses are needed when it is important to link the numbers of seed eaters to the number of seeds eaten and for studies that seek to determine the proportion of seed loss attributable either to different causes or among specific seed predators. Censuses are usually done before the study—to establish a baseline—as well as during the study. For established census techniques, see von Trebra (1994) and Sullivan (1979a) for small mammals; Franzreb (1981) and Millikin (1992) for birds; and Hulme (1994) for invertebrates. Von Trebra (1994), Sullivan (1979a), and Gashwiler (1970) also incorporated surveys of animal populations into research on conifer seed predation.

Observations about possible seed predators in the study area are also important in helping to determine the approach and methods used to study seed predation. For example, different techniques will be required to study animals that eat seeds on the tree as opposed to those that eat seeds on the ground.

5.3 Approaches to Studying Seed Predation

Studies of seed predation can be divided into those that examine predation on natural seed crops or on artificially introduced seeds. Studies of natural seed crops can focus on either pre-dispersal (in the tree) or post-dispersal (on the ground) predation. Predation on artificially introduced seeds can be studied using either unmarked seeds or seeds marked with paint.

5.3.1 Natural seed crops versus artificially introduced seeds

Natural seed crops provide seeds of natural abundance, species composition, and distribution in both time and space. They do not alter the behaviour or populations of the seed predators being studied (beyond normal fluctuations). Studying predation on natural seed crops is appropriate to examine predator–prey interactions, to avoid confounding the results with unnatural predator responses, and to study natural regeneration without artificial seeding. Introduced seeds may artificially increase the food source beyond natural background levels, which could elevate predator numbers or alter their behaviour in other ways. The quantity of available food will also be increased if only filled seeds are used (not all natural seeds will be filled).

Natural seed crops are required for studies of pre-dispersal seed predation, as no techniques for artificially attaching seeds to trees have been documented to date. Using natural sources of seeds also enables researchers to separate pre-dispersal from post-dispersal predation. However, studies of natural seed crops require the additional preliminary step of determining the species and numbers of seeds available, which is not necessary when using introduced seeds.

Artificially introducing seeds is probably the best approach for experimental research because the investigator can start with the same number and species of seeds in each experimental unit. This is also the obvious approach for studying the effects of seed predation on reforestation through artificial seeding. The spatial variability and unpredictability of natural seed supply would require a larger sample size and preliminary surveys (seed trapping) to determine an adequate sample size for the experimental design. Because seeds can be introduced artificially at any time, this approach is also appropriate for research on the time of year that seeds are least or most vulnerable to predation. Researchers can also use both natural and artificially introduced seeds to study different aspects of predation in the same project.

For exclosure-type studies, there may be a conflict between the size of seeds and the mesh size of devices used to exclude predators, particularly birds. Seeds that are too large (e.g., Garry oak acorns, winged maple seeds) to pass through the mesh required to exclude predators are probably best studied using artificially introduced seeds placed inside exclosures. For smaller seeds (e.g., most British Columbia tree species) mesh size should not present a problem unless fine mesh is used to exclude invertebrates. Although the screen can be removed during seedfall, it would be difficult to be sure that was no predation occurred during this period.

5.3.2 Predation on natural seed crops

Studying predation on natural seed crops involves first determining the species and numbers of seeds available for predation. Pre-dispersal seed numbers may be estimated by counting the number of seeds, cones, or other secondary reproductive structures on representative trees in the study area. Such estimates must be done before any predation begins and are essential for studies of pre-dispersal predation. As with all estimates, the problems associated with error must be taken into account (see Section 3.2 for methods used to estimate natural seed production). See Figure 3.12 for an example of the life table approach.

Pre-dispersal estimates can also be used to establish the baseline for post-dispersal (ground) predation studies, but trapping seeds on the ground is enough to give an estimate of the number of seeds available for ground feeders (see Section 4.3 for detailed methodology on seed trapping). If trapped seeds are compared with pre-dispersal estimates, it is possible to separate seed predation (or at least seed loss) occurring before and after seed dispersal.

Pre-dispersal predation can be assessed by comparing the estimated number of seeds or reproductive structures (e.g., cones) on selected trees before and after predation (e.g., see West 1989). This is probably the easiest method to use, but the error associated with estimating seed numbers may be high. Another method is to compare estimates of the number of seeds or reproductive structures on the tree before predation with the number of seeds collected in seed traps on the ground—after dispersal but before ground predation. This method assumes that predation is the only source of seed loss before dispersal. It is also theoretically possible to place exclusion devices (e.g., screens or cages) around certain branches to prevent predation. Seed predation can then be assessed by comparing seed number

estimates on protected branches with numbers on unprotected branches. We have found no documented examples of this last method.

Post-dispersal predation is usually determined by comparing the number of seeds or germinants found on the ground in an area that is subject to predation, with the number of seeds or germinants found in a comparable area where predation has been excluded. Approaches to exclusion are discussed in more detail in Section 5.3.3 under *Unmarked seeds*. The numbers of seeds before and after predation in fixed areas without exclusion could also compared. This approach does not allow any assessment of the number of seeds lost to other causes.

5.3.3 Predation on artificially introduced seeds

The first step in a study on artificially introduced seeds is to distribute the seed. This can be done from the air over large areas or by hand over smaller areas.

Large-scale distribution is used for operational seeding and this method can be used for research on the impact of predation on artificial seeding for regeneration. Seed distributed this way is similar to naturally dispersed seed in that the researcher has no control over the number of seeds per experimental unit, although the spatial distribution of seeds may be less variable than with natural dispersal. Seed traps can be used to evaluate seed distribution following aerial seeding.

Smaller-scale hand seeding is more labour-intensive but better suited to studies requiring tight experimental control, because the same numbers of seeds can be placed in each experimental unit. With hand seeding, there are two possibilities:

1. unmarked seeds can all be distributed at the beginning of the study and the remaining seeds counted one or more times, potentially until no more seeds are found; or
2. the location of each experimentally distributed seed can be marked (e.g., with toothpicks) and the missing seeds replaced at each monitoring date.

A single input of seeds may more closely mimic natural seedfall patterns (and therefore predator response) and is less time consuming. Replacing seeds is more labour intensive, but the pattern of seed consumption could be determined without the effects of declining seed numbers (i.e., because predators may make less effort as seed numbers go down and therefore predation would decline at a different rate). An advantage of marking seed locations, even without replacement, is that it could help determine how many seeds are lost to other causes, such as germination or rot.

Both marked and unmarked introduced seeds can be used to help determine loss to different predators and to causes other than predation, such as being unable to relocate seeds. Glass beads, the same size as the seeds being studied, have been used to determine the size of the loss due to the inability to relocate seeds (Johnson and Fryer 1996).

Unmarked seeds

Experiments that use unmarked seeds are relatively simple to set up and require no specialized equipment or materials. For these reasons, more seeds can be monitored for the same effort, which can improve sample size. However, naturally dispersed seeds must be excluded or background seed rain recorded to separate unmarked experimental seeds from naturally dispersed seeds.

Unmarked seeds can be studied both with and without exclosures, employing methods similar to those used for studying post-dispersal predation on naturally dispersed seeds. Distributing unmarked seeds in defined areas and then counting the survivors one or more times over one or more seasons is the simplest procedure. However, to determine other causes of seed loss or to distinguish among predators (when using unmarked seeds) it is necessary to selectively exclude different categories of predators (e.g., birds, small mammals, invertebrates). With selective exclusion of predators, unmarked seeds can be used to distinguish among broad predator groups, but not among individual predator species.

Different predator groups can be physically excluded from seeds with different types of cages (exclosures). Consumption can be compared among different exclusion devices and with no exclusion to assess the proportion of seed loss attributable to each group. Studies by Gashwiler (1970) and Hulme (1994) are good examples of this approach. Excluding all predators may provide some information on other types of seed loss, such as germination or decay.

Marked seeds

Seeds can be marked with either paint or radioisotopes. Experiments that use marked seeds can provide more complete information about causes of seed loss because the recovery rates are generally high (Lawrence and Rediske 1962; Fraser 1975) and individual predator species can sometimes be distinguished by distinctive marks left on the seed remains. The location in which a recovered seed is found may also provide clues to what ate it (for example, seed remains found in the burrow of a particular species). Recovering marked seeds is time consuming, and therefore not suited to studies that require a large number of seeds.

Radioactive marking requires highly specialized equipment and facilities and the laboratory and operators must be properly licensed to handle radioactive material. However, at the appropriate concentration, radioisotopes do not appear to affect germination of tagged seeds and yield the highest recovery rates (Lawrence and Rediske 1959).

Other substances used for marking seeds include plastic paints of various colours (Liddle et al. 1987), invisible fluorescent paints (Colbry 1967), and Day-Glo fluorescent dyes (Fraser 1975). Although paints do not involve the same difficulty in handling as radioisotope markers or require specialized recovery tools, neither the effect of paints (plastic and fluorescent) on germination nor their resistance to abrasion is known. Latex, used to bond fluorescent dye to seeds, provides resistance to abrasion but will also lower germination rates (Fraser 1975). Predators might also be repelled or attracted by the paint and thus bias estimates of predation (e.g., birds see and respond to colours), but there is no information available on this potential problem. The technique may also be unsuitable for tiny seeds. Although recovery rates are good, they are probably not as high as radiotagged seeds for the same amount of effort.

5.3.4 Quantifying seed predation

Regardless of the approach taken, predation can be quantified by counting seeds or germinants, either once or many times after predation. Counting numbers of seeds is the most common procedure, but could result in overestimating predation because losses due to other causes, including germination, will be counted as "eaten." Marking seeds or locations of seeds will help alleviate this problem. Counting germinants only may underestimate predation since some ungerminated seeds could escape predation and germinate later. A combination of approaches allows the researcher to separate seed loss to germination compared to other causes (including predation) (see Johnson and Fryer 1996).

The simplest way to quantify seed predation is to count seeds before and once after predation within a single year. This approach is useful for pilot studies to provide a broad estimate of predation and for making quick comparisons among several treatments or sites. However, both tree seed crops and predator populations can fluctuate significantly within and among years, so results from single counts cannot be extrapolated to other seasons or years.

Monitoring seed loss several times within one year can provide information about the seasonal vulnerability of prey seeds. Examples of factors that can affect seasonal seed predation rates include the abundance of other, preferred food sources in summer, and the absence of migrating bird species in winter. If seeds are trapped for monitoring predation on natural seed crops, patterns in vulnerability can be correlated with patterns in seed availability. The frequency of monitoring has varied in previous studies from two or three samplings (Gashwiler 1967, 1970) to weekly samplings for several months (Lawrence and Rediske 1962). The former focused on specific periods, such as overwinter survival of seeds, while the latter was able to follow the precise fate of seeds tagged with radioisotopes.

Multiyear sampling is essential for studying predator–prey interactions, predicting the long-term patterns of predation, studying the effects of disturbances such as forest harvesting, and determining the impact of predation on tree regeneration from seed. As with single-year studies, monitoring frequency within any one year can vary.

5.4 Methods, Techniques, and Equipment

See Sections 2 and 3.1 for information on surveying natural seed crops.

5.4.1 Distributing seeds

Large-scale distribution of seeds can be accomplished with either a fixed-wing aircraft or helicopter fitted

with various types of seeders (Mitchell et al. 1990). The quantity and uniformity of seed distribution are controlled by the calibration of the seeder and method of flying. Larger areas can also be operationally planted on the ground using seeders attached to machines that create scarified planting spots or furrows. This method requires fewer seeds than aerial seeding.

5.4.2 Excluding seed predators

The choice of exclosure design and materials depends on which predator group or combination of predator groups you want to exclude. Table 5.1 summarizes the exclusion choices for each of the three main predator groups and combinations of predators. Ground-feeding birds and small mammals can be prevented from gaining access to seeds with cages made of wire mesh (hardware cloth) on wooden frames, with or without sheet metal. Invertebrates can be excluded with fine mesh or sticky barriers, or can be removed from selected areas with chemical poisons.

Similar designs can be used for both birds and small mammals, but bird exclosures must have mesh over the top, whereas small mammals can be excluded with open-topped designs as long as the walls are tall enough and of materials the mammals cannot climb (e.g., sheet metal). The walls of small mammal exclosures must also extend below ground, to prevent the animals digging their way in.

Exclosures may be square, rectangular, or cylindrical, and enclose areas from 0.37 to 20 m^2 and be from 0.6 to 1.7 m tall. Mesh sizes smaller than 1 cm will effectively exclude small mammals (Wagg 1964; Gashwiler 1967; Sullivan and Sullivan 1982). Mesh size can be larger for birds, but 2.5 cm may still allow several bird species such as juncos, chickadees, and small finches to enter (Gashwiler 1970). Therefore, to exclude bird species but allow entry by small mammals, mesh sizes >1.0 cm but <2.5 cm are recommended.

The wood frame seed trap (described in Section 4.3.2) can be adapted as a vertebrate exclosure where a design with a lid is required. This design allows for easy access to the exclosure to count seeds and/or germinants. However, unlike seed traps, exclosures should not have window screening on the bottom, as this could impede germination of seeds and requires removal of all vegetation in the exclosure.

Exclosures designed to exclude only small mammals have sides made of a strip of wire mesh topped with a strip of sheet metal and no lid. The height of the sides depends on the type of small mammal to be excluded. Bending the sheet metal outward helps to prevent the animals from getting in (Figure 5.1). Joints between pieces of sheet metal must be smooth and tightly sealed since chipmunks have been able to climb exclosure walls by getting a toehold in the seams (Wagg 1964). Vegetation that the animals could climb to get into the excluded area must also be removed on a regular basis. The depth to which sides are buried will depend on how soft the soil is and what species of predators are in the area. Walls buried 10 cm deep have successfully prevented invasion, but even burial up to 30 cm may still allow entry of some animals (Gashwiler 1970). Sheet metal could alter the microclimate within smaller exclosures, so larger areas are recommended for such designs. Seeds could then be placed in the centre of the exclosure to avoid microclimate edge effects. Wire mesh allows the entry

TABLE 5.1 *Summary of exclosure choices for seed predators*

Predator(s) to be excluded	Exclusion device
Birds	Wire mesh cage with mesh lid, mesh >1 cm and <2.5 cm
Small mammals	Wire mesh and sheet metal cage with no lid, sides buried
Invertebrates	Pesticides and/or sticky barriers
Birds and small mammals	Wire mesh cage with mesh lid, mesh <1 cm
Birds and invertebrates	Lidded mesh cage (as above) with pesticides/sticky barriers
Small mammals and invertebrates	Wire mesh and sheet metal cage (as above) with pesticides/sticky barriers
All three groups	Very fine mesh cage with lid and sides buried

of seeds from natural sources and should not alter microclimatic conditions inside the exclosure.

Flying invertebrates can only be physically excluded with a fine mesh (e.g., window screening). Therefore, such exclosures are only practical for studies using artificially introduced seeds, because most seed from natural sources would also be excluded. Flightless insects (such as ants) can be prevented from gaining access to seeds placed on an elevated platform by coating the underside of the platform with a sticky substance, such as Tanglefoot™ (Heithaus 1981). Sticky strips could also be laid around the perimeter of the excluded area, although they would have to be checked regularly to remove debris that might provide a "bridge."

Chemical poisons have also been used to separate invertebrate predator groups from each other and from vertebrate seed predators. Note, however, that poisoning small mammals for this purpose is not legal in British Columbia (Mitchell et al. 1990), and in any case is considered ineffective because reinvasion is rapid (Sullivan 1979b; Mitchell et al. 1990). Because

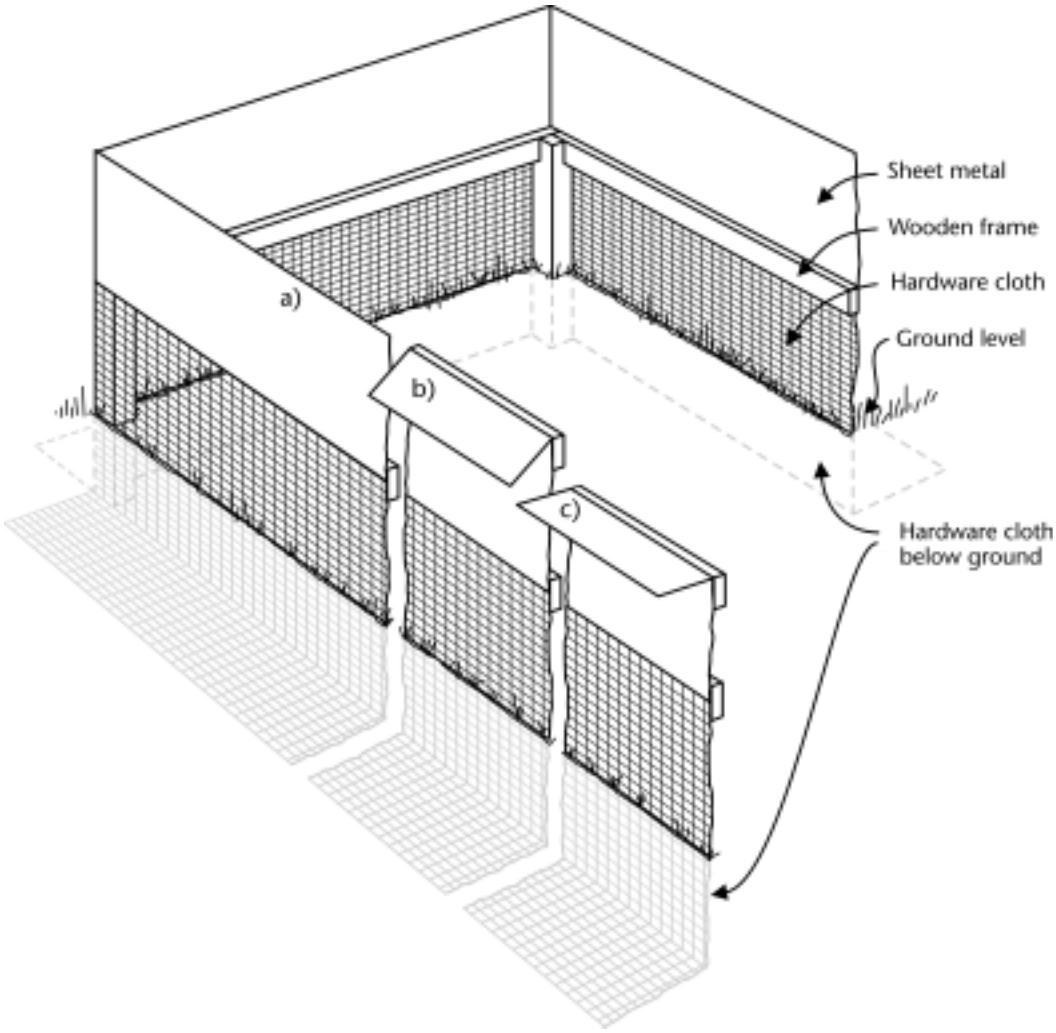


FIGURE 5.1 *Small mammal exclosure described by Wagg (1964). The three illustrated wall designs show: (a) Simple vertical wall, (b) hardware cloth wall with sheet metal bent downward to the outside, and (c) hardware cloth and sheet metal wall with top bent horizontally to the outside and supported by additional wooden frame. Different widths of sheet metal and hardware cloth should be tested; however, Wagg recommended that the sheet metal be at least 45 cm wide for designs a) and c) and that the bent portion be at least 15 cm wide.*

of the potential for poisoning non-target species we do not recommend using chemical exclusion.

5.4.3 Marking and recovering seeds

Radiotagged seeds

In Canada, use of radioisotopes requires licensing from the Atomic Energy Control Board of Canada. Proposals are reviewed to determine how the radioisotope is being used, how often it is used, what the concentration is, whether it occurs naturally, and what the half life is (C. Smith, Univ. B.C. Radiation Safety Officer, pers. comm., 1997). An environmental review, specific provisions for disposal of the material, or amendments to an existing licence for particular uses may be required before approval is granted. Research that uses low concentrations of a short-lived isotope (especially a naturally occurring one) will be subject to fewer restrictions and difficulties in obtaining approval. It should be noted that federal regulations concerning use of radioactive materials are being rewritten.

In several older studies, the radioisotope Scandium^{46} was used to tag seeds because it is a strong gamma emitter, has a half-life of 85 days, has low solubility, and is not phytotoxic. This half-life is considered short and the concentrations used (3 microcuries per seed) very low. However, there may be isotopes available that pose a lower risk to the environment and could be adapted for this application using the methods, summarized below, from the older literature. We have not found any recent published accounts of the technique.

Scandium^{46}, in the form of $ScCl_3$ solution, is first diluted to a working solution of 516 microcuries/mL (Lawrence and Rediske 1959). A drop of the surfactant Tween-203 added to the working solution increases penetration of the seed coat by the radiotracer. The tagging procedure is standardized by soaking 200 cleaned seeds for 1 hour in 5 mL of diluted Sc^{46} solution. Seeds are removed and air dried for 24 hours. The initial activity of the tagged seeds should average about 3 microcuries per seed.

Sc^{46} at a concentration of 3 microcuries per seed does not appear to impair the germination percentage of tagged seeds (Lawrence and Rediske 1959). Before placing seeds in the field they may be slightly moistened with a 3% Rhoplex solution to minimize any loss of the tracer through weathering.

A sodium iodide crystal scintillator is used to relocate radioactively tagged seeds in the field (Lawrence and Rediske 1959; Radvanyi 1966). Portable scintillometers are designed for rugged use and are battery powered with a hand probe. A Geiger-Mueller counter is not sensitive enough to gamma radiation to be effective. The range of the scintillator is about 80 cm in air and 45 cm through soil.

To locate tagged seeds or hull fragments, either a dial reading or earphones can be used. With the probe held approximately 30–45 cm away from the radiating source, detecting whole seeds or larger seed coat fragments is quite easy even 3 months after placement of the seeds (Radvanyi 1966). When a seed is missing from the point of initial placement, a spiral search pattern with the scintillometer probe should be conducted until the seed is found.

Painted seeds

Plastic paints and Day-Glo fluorescent dyes can be seen in daylight. Day-Glo pigments fluor-esce under visible wavelengths such as violet, blue, and blue-green (Fraser 1975). Invisible fluorescent paints appear white in daylight but require a source of longwave ultraviolet light to cause fluorescence.

Both plastic and invisible fluorescent paints can be sprayed onto seeds (Colbry 1967; Liddle et al. 1987). The D-series of Day-Glo pigments has increased stability in direct sunlight and can be applied using the methods of Fraser (1975) by: (1) immersing seeds for 2 minutes in a constantly stirred, 20°C solution of 5.5 g powdered dye in 10 mL acetone, then removing and air drying on paper towel, or (2) immersing seeds until completely coated in a 1:9 solution of Dow Latex 512-R and distilled water (constantly stirred), removing seeds and placing in a constantly agitated container of powdered dye until thoroughly coated. Excess dye can be removed by screening. The coated seeds are then air dried on paper towels. The second method makes the dye very resistant to weathering, but does reduce germination.

Recovery of painted seeds is labour intensive and time consuming, and involves sifting through the site for seeds. Locating seeds marked with invisible fluor-

escent paints is also a difficult task, and a longwave ultraviolet light source is required for recovery. Organic dyes such as Day-Glo fluorescent pigment fluoresce under daylight conditions and recovery of marked seeds can be as high as 98% (Fraser 1975).

Interpreting marks on seed remains

Marks left on seeds fed to specific captive predators can also be compared to marked and recovered seeds. Redpolls and chickadees slit the seed coat and remove the embryo of birch seeds (J. Zasada, pers. comm. 1997). For illustrations and descriptions of marks left by other specific predators, see Lawrence and Rediske (1959, 1962) and Radvanyi (1966, 1970). Radvanyi (1966) described marks made by captive predators of white spruce seed as follows:

- Mice and voles generally remove a third to half the seed coat on one side of the seed and consume the entire endosperm and embryo. The edges of the remaining seed coat are scalloped but entire.
- Chipmunks chew the seed in half, usually with the plane of the cut edge at right angles to the long axis of the seed. The chewed edges of these seeds tend to splinter and are more deeply cleft than those eaten by mice.
- Shrews generally make a smaller opening in the seed coat than do mice and chipmunks. The chewed edges are more finely serrated and the endosperm incompletely removed.
- Insects exhibit considerable variation in the size and nature of the opening in the seed coat.

5.5 Data Analysis

Much of the information about experimental design and sampling considerations discussed in seed dispersal studies (in Section 4.4) applies, with some adaptation, to seed predation studies.

Analyses for seed predation studies general seek to:

- estimate the proportion of seeds lost to seed predators; and
- compare these proportions among different seed species, different groups of predators, or different site conditions.

The proportion (p) of seeds lost to predators over time can be estimated from the number seeds on the ground before and after predation. An estimate of p is given by the formula:

$$\hat{p} = 1 - \frac{\text{number of seeds remaining}}{\text{number of seeds available}} .$$

The approximated standard error of this estimated proportion is given by the equation

$$S.E.(\hat{p}) = \sqrt{\frac{\hat{p}(1 - \hat{p})}{n}} ,$$

where n is the number of seeds available.

Example

To compare the proportion of seeds of the same species taken by two different groups of predators, use either a z-test when n is large (both $n(p)$ and $n(1\text{-}p)$ must be greater than 15 for the two sets of predation data), or a contingency table chi-square (χ^2) test.

Let $\hat{p}_1$, n_1 denote the proportion of seeds eaten by and number of seeds available to predator 1, and $\hat{p}_2$, n_2 the same for predator 2. The estimated difference between the proportions of seeds lost to predator 1 compared to predator 2 is $(\hat{p}_1 - \hat{p}_2)$, with the approximated standard error

$$S.E.(\hat{p}_1 - \hat{p}_2) = \sqrt{\frac{\hat{p}_1(1 - \hat{p}_1)}{n_1} + \frac{\hat{p}_2(1 - \hat{p}_2)}{n_2}} .$$

To test the null hypothesis that the difference between these two proportions is zero versus the alternative hypothesis that the difference is not equal to zero, use the z-test with the test statistic,

$$z = \frac{\hat{p}_1 - \hat{p}_2}{S.E.(\hat{p}_1 - \hat{p}_2)}$$

when the sample size is large. The computed test statistic is compared with tabulated values from the standard normal table (z-table). The null hypothesis (of no difference) is rejected if $|z| > z_{\alpha/2}$ where $z_{\alpha/2}$ is the tabulated z-value corresponding to a cumulative probability of $(1 - \alpha/2)$.

If the large sample size requirement for the z-test is not satisfied, then the contingency table approach can be used to make comparisons. In this case, the

actual seed counts are used as the data rather than the proportions lost and remaining. Table 5.2 shows the data structure.

TABLE 5.2 *Two-dimensional contingency table for analyzing seed losses to two predators*

	Number of seeds not eaten	Number of seeds eaten	Row total
Predator 1	n(1, not eaten)	n(1, eaten)	n_1
Predator 2	n(2, not eaten)	n(2, eaten)	n_2
Column total	n(not eaten)	n(eaten)	N

where: n(1, not eaten) = number of seeds not eaten for predator 1
n(1, eaten) = number of seeds eaten for predator 1
n(2, not eaten) = number of seeds not eaten for predator 2
n(2, eaten) = number of seeds eaten for predator 2
$n(1, \text{not eaten}) + n(1, \text{eaten}) = n_1$
$n(2, \text{not eaten}) + n(2, \text{eaten}) = n_2$
$N = n_1 + n_2$ = total number of seeds available

The chi-square test can be used to compare the distribution of the seed counts across the two categories for the two predators. The chi-square test statistic is given by

$$\chi^2 = \sum_{\text{all cells}} \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

where *observed* represents the observed count in the sample, and *expected* represents the expected count. The expected counts can be computed by multiplying the corresponding row and column totals, and then dividing by the grand total. For example, the expected number of seeds not eaten for predator 1 is:

$$E(1, \textit{not eaten}) = \frac{n_1 \times n(\textit{not eaten})}{N} .$$

The observed χ^2 value is compared with a tabulated value, χ^2_α having degrees of freedom equal to (number of rows -1)(number of columns -1).

Both the *z*-test and the contingency table chi-square test can be used to compare the proportion of a particular species of seed lost to several different predators in two different site types (e.g., different site series, logged versus unlogged). The contingency table approach is suitable for comparing more than two predators and more than two site conditions (e.g., three or more site preparation treatments, or seral stages). One requirement of the chi-square test is that the expected count in each cell must be greater than 5.

To compare more than two factors, a log-linear model which is a generalized approach to analyzing multidimensional contingency tables can be used. For further discussions on categorical data analysis methods, see Agresti (1990), Chapter 14 of Johnson and Bhattacharyya (1992), and Lesperance (1996).

When monitoring seed predation behaviour over time, two different methods can be used to measure the proportion of seeds lost to predation:

METHOD 1 Measure the proportion based on the initial number of seeds available and the number of seeds remaining at each measurement time. These are, in fact, cumulative proportions. The resulting time plot will have a decreasing pattern. The data obtained from this method can be used to determine how much seed to put out to have adequate germination for regeneration.

METHOD 2 Measure the proportion based on the number of seeds available at the beginning of each time period and the number of seeds remaining at the time of measurement. The resulting time plot will not necessarily be decreasing. It will likely have a cyclical pattern, showing the times at which predation is at its highest or lowest. These data can be used to determine the optimal time for releasing seeds for best germination results.

Table 5.3 illustrates the two methods for computing predation proportion over time using hypothetical data.

TABLE 5.3 *Comparison of methods for calculating proportion of seeds lost to predation over time*

Time	Number of seeds not eaten	METHOD 1 cumulative proportion eaten	METHOD 2 proportion eaten per time period
0	100	0	0
1	90	0.1	0.1
2	72	0.28	0.20
3	66	0.34	0.08
4	53	0.47	0.20

SECTION 6 SEED BANKS

The wingéd seeds, where they lie cold and low,
Each like a corpse within its grave, until
Thine azure sister Spring shall blow.
(Percy Bysshe Shelley "Ode to the West Wind")

6.1 Background

Many of the viable seeds produced by trees fall to the ground, become buried in the soil, and do not germinate for several years. Seeds of other species may be retained by trees for many years before they are released and germinate. These dormant, viable seeds, stored in either the soil or in tree canopies, are called seed banks. This chapter deals with soil seed banks only; canopy seed banks are discussed in Section 3.5.1 because the methodology is similar to that used for pre-dispersal surveys.

Knowledge of the species composition, numbers, and distribution of seeds in soil seed banks is important for understanding and predicting natural regeneration and revegetation after disturbance; developing effective vegetation management prescriptions; and describing overall floral diversity.

Seed banks of tree species in northern temperate and boreal forests, such as those found in British Columbia, have not been studied extensively. According to Archibold (1989), conifer seed viability may last from 1 to 5 years under controlled conditions, and perhaps as long as 10 years for white spruce (see Section 2.1). The longevity of angiosperm tree seeds in artificial storage (freezers and refrigerators) ranges from a few months to decades, depending on the species and storage conditions. For more details see Schopmeyer (technical coordinator, 1974).

Although there has been much research into optimum artificial storage conditions to maintain seed viability, little is known about how long tree seeds remain viable under natural conditions (i.e., either on or buried in forest floor). Recent results from a seed burial study on southern Vancouver Island (C.L. Leadem, unpub. data 1997) suggest that the seeds of many British Columbia conifer species and some hardwood species germinate within the first growing season when buried outside. A few hardwoods (e.g., alder and birch species) persisted for up to 2 years after burial and may last even longer. Seeds of pin cherry (*Prunus pensylvanica*) are known to remain dormant in soil for several decades (Marks 1974). In contrast, the viability of seeds of *Populus* and *Salix* species lasts from only a few hours to a few weeks (Haeussler et al. 1990) and, therefore, these are not considered seed bank species.

Questions that might be asked in seed bank studies include the following:

- What species are represented in the seed bank?
- How many seeds of each species are present in the seed bank?
- How many of the seeds are viable?
- How many of the seeds found in seed banks will germinate (1) under controlled conditions and/or (2) in the field under natural conditions?
- What is the vertical distribution of seeds?
- What is the horizontal distribution of seeds?
- How is seed distribution in the soil related to seed source?
- How is germination of seeds affected by disturbance?
- How long can seeds remain viable in the soil?
- What conditions break the dormancy of buried seeds?

6.2 Approaches to Studying Soil Seed Banks

The most common approach to studying existing seed banks involves collecting soil samples from the area of interest. Species, numbers, and distribution are then determined either by separating the seeds from the soil and directly counting and identifying them (direct count method), or by allowing seeds to germinate from the soil samples in a controlled environment and counting and identifying the germinants (sample germination method). With either direct counts or sample germination, vertical distribution can be assessed by dividing soil samples into layers parallel to the soil surface.

Two additional approaches have seldom been used but are essential for meeting some of the above objectives. Monitoring germination from seed banks in the field is used to determine the number and species of seeds that will actually germinate under field conditions and thus contribute to post-disturbance regeneration (Yearsley 1993). Studying seed mortality and the duration of dormancy under natural conditions in the soil requires burial of known quantities of seed which are retrieved at regular intervals and tested for viability (e.g., Granstrom 1987). These two approaches can be used together to enhance the information collected. Other approaches to studying germination are discussed in Section 7.2 (seed germination under controlled laboratory conditions) and Section 7.3 (seed germination under field conditions).

6.2.1 Seed separation versus direct counts

Direct counts are appropriate when it is important to do a complete inventory of seeds present including viable and non-viable seeds. Sample germination is more suited to pilot projects where a rough estimate of the number and species of viable seeds is needed. Both techniques are appropriate when the objective is a baseline inventory of numbers and species composition. The most thorough approach is to first separate seeds from the soil, count them, and then germinate the separated seeds. Another approach is to separate seeds from the samples after a period of germination.

Direct counts yield the most accurate measure of the species and numbers of seeds present in the soil seed bank (see Brown 1992). This method also provides data on the number and species of non-viable seeds present. However, the seed separation process for direct counts is extremely time consuming, especially where the density of seeds is low. Also, to determine how many seeds are viable, additional tests must be done (e.g., tetrazolium stain). Often a high proportion of seeds are non-viable, so a large number of seeds must be tested to get an accurate estimate of the viable proportion. In addition, small seeds may be missed or hard to identify.

With sample germination, only viable seeds are counted. This method is less labour intensive than direct counts and can yield good results when the objective is to determine the number and species of viable seeds present in the soil (Gross 1990). Some species are more easily identified as plants (germinants or seedlings) than as seeds. It takes longer to get results from sample germination because the monitoring period required is at least 6–12 months. The main problem with sample germination is that some species and individuals will probably be missed, because their germination requirements are not met. Thus, seed numbers can be underestimated and species composition biased. Finally, the number and species composition of ungerminated seeds (whether viable or non-viable) cannot be determined with this method.

6.2.2 Assessing vertical distribution

For vertical distribution, layers can correspond either to the functional layers of forest floor (i.e., LFH layers: litter, fermentation, and humus layers) or to layers of equal thickness. Functional layers can also be defined simply as forest floor (organic layers) or mineral soil. The functional division will provide information that can be linked to other soil biological processes such as rates of organic matter accumulation and decomposition, soil fauna (including seed predators), soil mixing, etc. These layers often vary considerably, however, so the volume of soil involved will have to be determined for each layer. Also, environmental conditions such as temperature may vary with depth. Equal-thickness layers are the best means to generate depth distribution patterns for seeds. These distributions can probably be more easily linked to environmental conditions and the division of samples is easier because no judgements about the boundaries between functional layers are required. However, because the LFH layers vary in thickness,

seeds from one functional layer may be compared to seeds from another (e.g., litter with fermentation).

6.2.3 Monitoring germination in the field

Monitoring germination in the field is used to determine what germinates from the seed bank under natural conditions (including various disturbance regimes), and/or the long-term contribution of the seed bank to natural regeneration. Field monitoring will not provide any information about non-viable seeds and viable seeds that have not germinated, and the information is likely to come only from seeds close to the surface. Also, with this method there is little control over conditions such as predation or unplanned disturbances. Field germination can be done in conjunction with environmental monitoring to determine the conditions that result in germination. Seed trapping should also be part of field germination studies to help separate the seed bank germinants from recent seed rain germinants. Monitoring should be carried out over several weeks to months per season, and for more than one season, to describe the seed bank flora, because conditions in any 1 year can have a profound influence on whether seeds germinate and survive as plants.

6.2.4 Seed burial experiments

The age of seeds found in natural seed banks cannot be determined. This is essential to understanding and predicting the length of viability and therefore the potential for germination. Burial experiments can be used to determine how long seeds can remain viable in the soil. Such experiments can never completely mimic natural conditions because the seeds are usually placed in a mesh bag to avoid confusion with naturally occurring seeds and for easy retrieval. Mesh bags may exclude invertebrate and other seed predators and thus bias estimates of seed loss in the soil. To avoid this problem, seeds could be radioactively tagged, buried without enclosing them in mesh bags, and retrieved using a scintillometer (see Section 5), but recovery of seeds may not be as complete.

6.3 Methods, Techniques, and Equipment

6.3.1 Collecting and preparing soil samples

Soil augers are best suited to collecting soil samples in grasslands or in the mineral layers of forest soils where there are few large roots and little or no coarse litter or other organic material at the surface (Figure 6.1). Augers do not work as well for collecting forest floor samples because there are too many roots. The auger may crush or tear samples because of the high content of partially decomposed organic matter, especially in the litter layer.

FIGURE 6.1 *Using a soil auger to remove a soil core for seed bank studies.*

A sharp serrated knife (e.g., a new bread knife) works well to cut samples of forest floor (Figure 6.2a). Pruning clippers will cut through the finer roots, and a small pruning saw and/or long-handled pruning loppers can be used to cut through larger roots. You may have to cut a wedge of forest floor away from one side of the sample to gain access to it, especially for samples thicker than about 10 cm (Figure 6.2b). Sample locations should be moved a few centimetres away from trees or stumps to avoid large roots that can take up a large portion of a small sample and bias the volume collected. Use a flat trowel or spatula to lift the samples out after the sides have been cut (Figure 6.2c). A Japanese garden knife is sturdy enough to lift the samples out, and is sharp enough to cut the soil. An alternative is to lift out a larger volume of soil than is actually needed with a sharp shovel and then trim the sample to the required size.

Square samples ranging from 10 to 15 cm square (Matlack and Good 1990; Brown 1992) are easy to handle and fit into standard inserts for greenhouse trays. Samples smaller than about 6 × 6 cm are diffi-

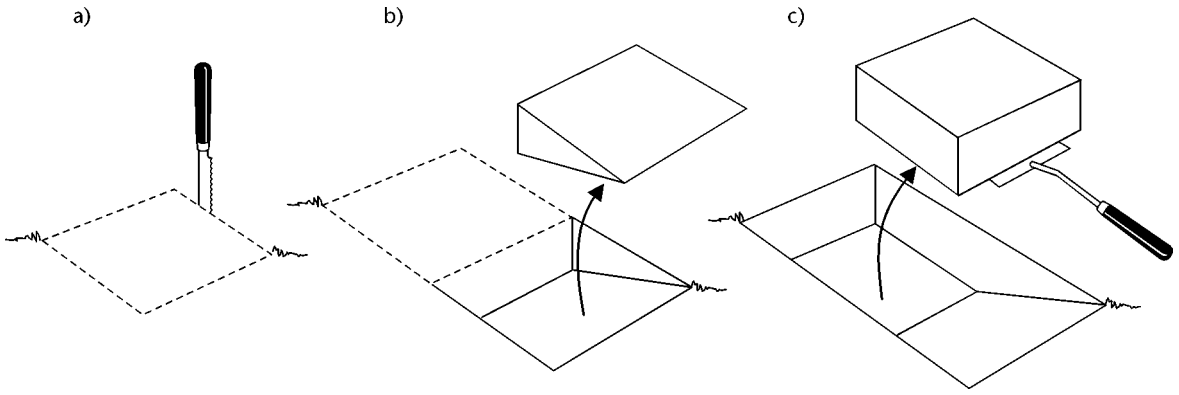


FIGURE 6.2 *Method for cutting a square forest floor sample: (a) cut the sides of the sample with a knife; (b) cut and remove a wedge of forest floor beside the sample; and (c) cut the bottom of the sample and lift out with a spatula.*

cult to keep intact unless they are thin (e.g., < 4 cm). The thickness of the sample may vary depending on soil thickness and the part of the profile of interest. Auger sizes range from about 2 to 10 cm in diameter; 8 or 10 cm are common sizes. They are capable of removing cores up to 20 cm thick.

Keep square samples intact until they are divided into layers by placing them directly into inserts and then into standard plastic greenhouse trays for transport. Avoid compressing samples unless vertical distribution is not important to the study. If the trays must be stacked, invert strong greenhouse trays over full trays to take the weight, or provide some other support for the upper layers of trays. Plastic sheets or remay must be placed between stacked trays to prevent seeds falling through the drainage holes into lower samples. If vertical distribution is not important or samples are divided into layers in the field (such as those collected with an auger), then the samples may be put into paper bags for transport. If plastic bags are used, allow for air circulation to prevent samples from going mouldy and killing seeds before they have a chance to germinate.

Samples collected in the summer and fall will contain seeds from the current year's crop. Samples collected in the spring will lack seeds that have germinated over summer or been lost to winter mortality (e.g., predation). If seasonal patterns of seed bank numbers are important, then samples should be collected in both spring and fall.

Samples collected for immediate germination in the spring do not need further treatment since they have just gone through winter *in situ*. Samples collected in summer or fall should be stratified for at least 4 weeks at 2–5°C. For more information about stratification of seeds or samples before germination, refer to Sections 7.2.1 and 7.2.3, and Tables 7.2 and 7.3.

If vertical distribution information is desired, cut the square samples into layers parallel to the soil surface (Figure 6.3). A sharp serrated knife and clippers make it possible to cut layers of forest floor as fine as 1 cm thick, if samples are small and moist enough to hold together. This is easier to do in the lab than in the field. Samples collected with an auger can be divided in the field by gradually pushing the sample out of the auger (Figure 6.4a), measuring the desired thickness, and slicing the layers off with a sharp serrated knife (Figure 6.4b).

6.3.2 Seed separation and direct counts

For direct counts, seeds must first be separated from the rest of the soil sample. Seed separation involves some or all of the following steps: dispersing or breaking up the soil sample, floating and removing larger pieces of organic debris, washing the remaining sample through sieves of various sizes, and floating and removing seeds (see Malone 1967; Benoit et al. 1989). A similar technique called elutriation uses a modified pneumatic root elutriator (designed to separate organic matter from soil) to separate seeds from the soil (Gross 1990).

Solutions of hexametaphosphate or Calgon (50 g/L) and sodium bicarbonate (25 g/L) are used to disperse or break up the soil sample (Benoit et al. 1989).

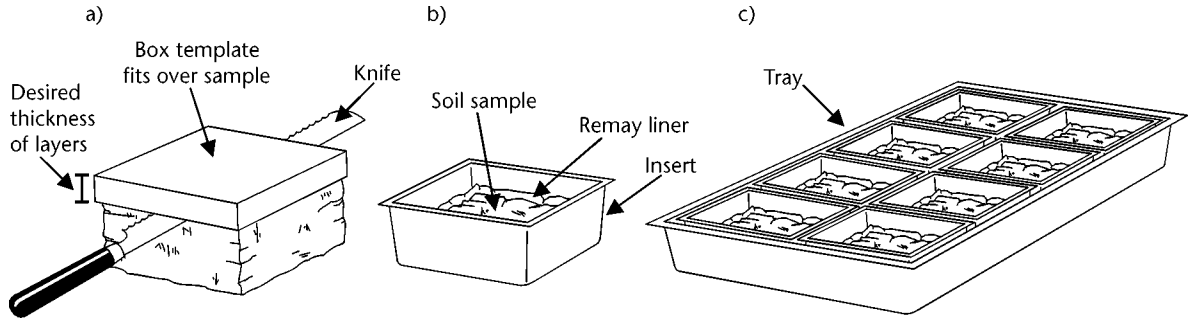


FIGURE 6.3 *Preparing square soil samples for greenhouse germination: (a) a sample is split into layers (parallel with the soil surface) using a cardboard box template as a guide to ensure the layers are of uniform thickness; (b) each layer is placed in an individual greenhouse tray insert; and (c) inserts are placed in greenhouse trays.*

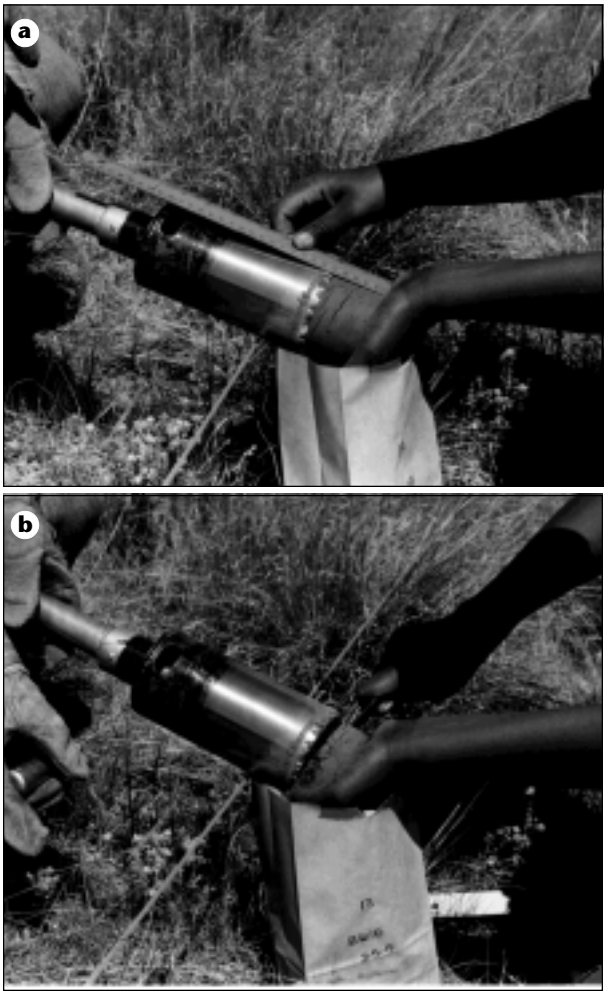


FIGURE 6.4 *Dividing a soil core into layers. For each layer the sample is pushed out of the auger and (a) measured, then (b) sliced off and placed in a labelled bag.*

Magnesium sulphate (Epsom salts) can also be added (Malone 1967). Soil separation may also be promoted by adding detergent to eliminate surface tension (J. Zasada, pers. comm., 1997). After the soil is mixed vigorously (2 minutes) or allowed to soak (30 minutes) in this solution, the organic material—including the seeds—floats to the top.

The suspended material is passed through one or more sieves to separate remaining soil and organic matter particles from the seeds. A single fine sieve can be used for a small volume of sample material, or a coarser sieve for large seeds. If you are trying to extract seeds of a particular species of known size, the mesh size of the sieves can be selected to maximize retention of seeds and exclusion of the other materials. For small seeds, a wide range of seed sizes or large volumes of sample material, samples should be washed through several mesh sizes (e.g., 2.0 mm down to 0.14 mm). The size of the finest-mesh sieve should be less than that of the smallest seeds (if known). The material left in the sieves is transferred to filter paper to dry for 24 hours.

Dried seeds are separated from the other debris by hand, using a dissecting microscope if necessary, and tallied by species. Reference collections of seeds are used to identify seeds (the University of British Columbia Botanical Gardens have some voucher specimens). The viability of most seeds can be determined using tetrazolium stain (described by Moore 1976). Seeds of species that do not stain reliably with tetrazolium may be tested for viability by germination in a growth chamber or other means. See Section 7.2 and Leadem (1984) for more information on seed viability tests.

6.3.3 Germinating seeds in samples

Samples are usually germinated in a greenhouse, but they can be placed in a growth chamber or kept outside. Because environmental conditions can be finely adjusted, a growth chamber is useful for controlled experiments or for testing seeds separated from soil samples. Growth chambers, however, are too small to be used for large volumes of soil. A greenhouse is essential if the samples are to be germinated in the winter. In the greenhouse, temperature can be kept relatively steady with a computerized system, or may fluctuate by up to 5°C using manual thermostats. Light and watering regimes can be maintained automatically with timers or computer control. Samples kept outside must be adequately protected from contamination by outside seeds. Outside germination conditions cannot be controlled, but it is still a useful way to monitor a large number of samples where greenhouse space is limited. If the location is close to the site where the samples were collected, conditions can be similar to those experienced by the intact seed bank.

Inserts in greenhouse trays are the most efficient way to fit samples into a given space (Figure 6.5). If space is not limiting, any size or shape of plastic pot will do as long as the samples are treated consistently (i.e., do not put some in deep pots and others in shallow pots). Samples may be mixed with or placed over a sterilized growing medium, such as potting soil, to provide extra soil for growth of germinants and to help prevent thin or small samples from drying out. The samples may be left intact or broken up. A remay liner in the container will prevent tiny seeds being lost through the drainage holes.

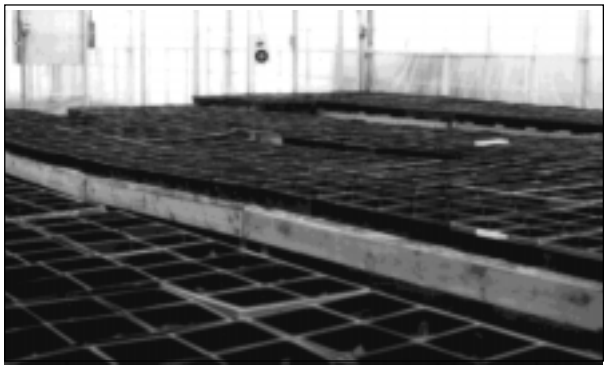


FIGURE 6.5 *Soil samples in the greenhouse. Standard plastic inserts and trays efficiently accommodate the large number of samples required to adequately characterize a seed bank.*

Samples should be randomly distributed in a greenhouse since conditions usually vary throughout the building. Rotating samples periodically will ensure that all samples experience all conditions. If possible, try to record the temperature manually in several different areas in the greenhouse to determine how much variation there is. You may have to adjust the watering regime if samples dry out at different rates in different areas.

Samples must be protected from contamination by wind-dispersed seeds, even in the greenhouse. Remay is an effective barrier against unwanted seeds but lets in light, air, and water. However, any barrier must be easy to remove for germination counts. Instead of a barrier, you can distribute trays of sterilized potting soil among the samples and estimate contamination by counting germinants in the potting soil.

Water the samples frequently (as often as once a day) as they may dry out quickly and both seeds and germinants are vulnerable to drought. During germination, samples should be kept above freezing and below 30°C. Temperature is often kept on a diurnal pattern that is warmer in the day than at night which can be synchronized with the light regime to achieve a specific daylength. Light supplementation may be especially important for samples germinated in the winter. If known, temperature and light conditions can be set to mimic natural daily and seasonal regimes, as these will give results most similar to field germination.

Germinants should be clipped off or pulled as soon a they have been positively identified. Some species are distinct even at the cotyledon stage and can be removed quickly. However, most germinants will need to be grown until they produce true leaves and, sometimes, reproductive structures, before they can be identified. New germinants that cannot be identified immediately must be marked to distinguish them from older unidentified germinants. Otherwise, new germinants may be mistaken for germinants that were counted on previous sampling dates but have since died, resulting in underestimated seed numbers. Mark each new germinant with a toothpick, a small loop of thread or wire, or a collar cut from a plastic drinking straw (J. Zasada, pers. comm., 1996), using a different colour for each sample date. Toothpicks are

suitable where there are only a few germinants spaced well apart. Thread or wire loops or straw collars work best where there are many germinants.

Another method is to transplant unidentifiable germinants as soon as they emerge. However, this approach includes a number of drawbacks: more space is required for the individual pots; more time is needed to transplant and care for all the germinants; ungerminated seeds could be removed from the sample with the soil around the transplants roots; and mortality may be high due to the transplanting process. Transplanting is recommended only for species that must be grown to a large size or for a long time before a positive identification can be made (see Franklin 1961).

The samples should be monitored frequently (two or more times a week), especially at the beginning of the germination period, until the rate of germination and mortality has been established. Besides drought, germinants can be lost to attack by fungus, mould, insects, and other pests. Frequent monitoring will help you observe potential problems so that solutions can be found before many seeds or germinants are lost.

After an initial flush, germination often slows down. Stirring, crumbling, or turning samples over can stimulate renewed germination by exposing ungerminated seed to light. Varying the temperature regime can also help, or samples can be put through another cycle of cold stratification. Samples should be monitored as long as seeds are still germinating, if possible. Several months will be required in most cases, and possibly more than one year.

6.3.4 Monitoring germination in the field

(See also Yearsley 1993 and Section 1, Table 1.1.)

The study site must be clearly marked on a map with written directions so that it can be easily relocated. Within the site each monitoring plot must also be marked. Depending on the layout, tall stakes (rebar or wood) painted and flagged can be used to mark the boundaries of larger areas enclosing several monitoring plots. Each corner of the monitoring plot should be marked with a short piece of rebar or large spike, painted and flagged at the top. An accurate map of the study site showing the locations of all the monitoring plots in relation to each other, and to important permanent site features, is also essential.

If only field monitoring is being carried out, then larger plots can be used to maximize the area sampled with fewer plots. Plots should still be small enough to allow for access without walking in them to look for germinants. The abundance of germinants should be taken into account, if possible, when deciding on plot size. In general, the more abundant the germinants the smaller the plot can be. Smaller plots will also be easier to track germinants when other vegetation is abundant. Larger plots are appropriate in areas with few germinants and sparse vegetation. For studies where soil samples are also taken to compare field results with the overall seed bank, the monitoring plots should be the same size, shape, and distribution as the soil samples. Vertical distribution may be gauged by scraping off layers of soil of known thickness and then monitoring germination.

Like greenhouse germinants, individual field germinants should be marked as they emerge. Loops of coloured wire or plastic drinking straw collars may be best because they are less likely to be eaten or removed by natural causes, and will not rot. It is also useful to map the location of germinants within the monitoring plot to help keep track of them and provide information about the horizontal distribution of germinants. If only the identity and number of germinants are of interest, then the germinants can be clipped off or pulled out as soon as they are identified. For studies on the contribution of the seed bank to natural regeneration, markers will have to remain on the germinants to track them over time. If so, the original markers will have to be replaced with larger ones as the plants grow.

The study site should be monitored frequently (two or more times a week) because germinant mortality can be high, especially during a dry, hot spell. Good access to the site is essential, otherwise monitoring frequency may be compromised. Monitoring should begin as soon as snow (if present) has melted and continue as long as new germinants are being found. Recording and monitoring soil environmental conditions (e.g., moisture, temperature) along with above-ground conditions (e.g., temperature, precipitation) will help to explain the response of buried seeds.

In the field, seed bank germinants must be distinguished from those of recent seed rain. Records should be kept of potential sources of seeds in the vicinity of the study area and of the timing of seed

dispersal, especially wind-blown seeds. Seed traps can also be used to estimate the amount and species of seeds in the current seed rain (see Section 4). Trap numbers, size, and distribution should be the same as the monitoring plots. Combining field monitoring with determining the seed bank contents of soil samples removed from the site, will provide information about which species are definitely not in the seed bank. Although barriers (e.g., remay) can be spread over the plots to exclude current seed rain, they may significantly alter natural conditions (e.g., raise temperatures). Both covered and uncovered plots can be installed but this will increase the number of plots required for adequate replication.

6.3.5 Seed burial experiments

Mesh or cloth bags made of nylon (e.g., nylon stockings), fibreglass screening, or other synthetic fibres can be used to contain replicates of seeds for burial. Natural fibres such as cotton are less suitable because they may rot if the seeds are to be buried for more than a few months. If necessary, seedlots can be protected from larger seed predators by enclosing each batch of bags in hardware cloth cages (Haywood 1994).

Batches of seeds to be exhumed on different dates should be buried far enough apart so that it is possible to dig up each one without disturbing the remaining batches. Seeds should not be buried much deeper than they are likely to occur under natural conditions (although this may not be known for some species). However, it is useful to contrast more than one depth, especially if environmental conditions (e.g., soil temperature) can be monitored at the same time. Most tree seeds in British Columbia are probably found within 5–10 cm of the soil surface.

Bags containing seeds can be buried by removing intact blocks of forest floor, placing a bag in the hole, and replacing the soil block (Granstrom 1987); or by digging holes and backfilling without trying to maintain soil integrity (Leadem 1995). To approximate natural conditions, seed containers should be in contact with the soil.

Seeds should be recovered at least once a year for several years. Estimates of the number of years to plan for can be based on what is known about seed longevity under artificial storage conditions. Exhuming and examining seeds at different times of the year can show how seed losses vary depending on seasonal conditions.

The causes of seed loss may be difficult to determine. Where germination has taken place soon after burial it may be hard to tell whether the seeds germinated or simply rotted away. Losses can also be caused by fungi, microorganisms, or predators that chew through the seed bags. Undamaged seeds can be tested for viability using a variety of standard methods (see Section 7.2 and Leadem 1984).

6.4 Experimental and Sampling Designs

6.4.1 Seed bank inventory studies

Seed bank inventory studies involve determining the number, species composition, and distribution of seeds in the soil. The sampling design employed depends on the distribution of seeds in the study area (or on what the researcher believes the distribution is). Except for the case where the researcher is interested in assessing the horizontal distribution of seeds, random sampling should be used. Random sampling is based on the notion that each soil sample has an equal probability of being selected, and selecting a soil sample in no way affects the selection of any other samples. Random sampling ensures that no systemic error is introduced into the data. For assessing horizontal seed distribution, systematic sampling—usually using a grid—should be used.

If it is known that seed distribution is different for different site characters, such as site series, then the study area should be stratified by the site character before sampling. Stratified sampling often produces more accurate estimates. The stratification criteria should be chosen such that the variability within a stratum is smaller than that between strata. Stratification also allows seed bank inventory by stratum.

If the researcher does not know the seed distribution or a suitable stratification criterion, then simple random sampling should be used for the entire study area. If the researcher knows a suitable stratification criterion, but is unable to stratify the study area before sampling due to lack of knowledge of the study area, then either simple random sampling with post-stratification or double sampling for stratification could be used. For both sampling schemes, a simple random sampling of the study area is first conducted. In addition to the sampling variable of interest, the researcher would also collect data on the stratifi-

cation variable. In the case of post-stratification, the data would then be stratified based on the observed values of the stratification variable. Post-stratification is helpful if the study area cannot be stratified before sampling; however, it will likely result in unequal sample size per stratum. In the case of double sampling, the data in the initial sample are classified into strata. A second sample is then selected from the initial sample using stratified sampling. In double sampling, the researcher can control the sample size in each stratum. See Thompson (1992) for a thorough discussion on various sampling schemes.

The number and distribution of seeds in seed banks is highly variable, so the larger the number of samples collected the more representative the results will be. A larger number of small samples are generally considered to represent the seed bank better than a few large (e.g., greater than 15×15 cm) samples (Bigwood and Inouye 1988; Benoit et al. 1989). Although a large number of large samples would be ideal, bigger samples are difficult to collect intact and almost impossible to slice into uniform layers. In addition, the volume of soil may be too great to process. Large samples can be collected by taking a series of smaller, contiguous samples. This technique allows for spatial distribution mapping at a fine scale. However, because the individual samples within a contiguous group are not independent, they cannot be used for frequency calculations. Another advantage of small samples is that they fit better between the areas that cannot be sampled in many forest soils, such as tree roots, rocks, and fallen logs. This results in fewer sample locations being moved to avoid these obstructions.

The number of soil samples collected depends on how accurate the estimate needs to be, how often the estimate can be wrong, how much variability is in the data, and what resources are available. The minimum number of soil samples can be determined based on the confidence interval formula generated from preliminary samples. Note, however, it may be impractical to collect and assess the seed bank content in these preliminary samples. The total volume of soil samples sufficient to be representative of a seed bank has been estimated to be 2000 cm^3 for pasture land (Forcella 1984), but for forest soils, sample volumes range from 5000 cm^3 (Brown 1992) to 155 000 cm^3 (Matlack and Good 1990).

A serious mistake in many previous seed bank studies has been to pool soil samples (combine and mix), then remove subsamples for seed bank determination. This practice results in losing information about variation among samples. Such variation is an important descriptor of the spatial distribution of buried seeds, and is essential to understanding the relationships between the distribution of seed sources and the distribution of seeds in the soil. In addition, this information will help future researchers choose appropriate sample sizes for forest soil seed banks, since so little is currently known. Data from groups of samples can always be pooled *after* collection from the original, individual samples.

For more discussion on experimental design, see Sections 1.4, 3.6, 4.4, and 7.3.1.

6.4.2 Comparison studies

Comparison-type studies require ample replication and randomization. Replication is an independent repetition of the experimental factor(s) and ensures that study results are not by chance. For example, if a study seeks to compare the number of viable Douglas-fir seeds in the seed banks in two different site series, then several locations per site series should be randomly selected for measurement. A location is an experimental unit for the factor *site series*. The number of replications is the number of randomly selected experimental units per site series. Within a location, multiple soil samples might be collected for measurement. These soil samples are called subsamples. A common mistake is to have a single location for each site series and regard the soil samples within a location as experimental units. This situation is called pseudoreplication, because the researcher assumes the experimental factor is replicated when in fact it is not (see Section 1.4). A design with pseudoreplication could indicate differences between the two locations, but no conclusion could be made about the two site series.

Randomization refers to the random assignment of the experimental factor to the experimental unit. It is a means for reducing systematic errors in the data. In the case of site series, it is not possible to randomly assign site series to a location—a location belongs to a certain site series before the experiment is conceived. To compensate, locations must be randomly selected from all possible locations available for the

experiment for a particular site series. Randomization also includes random selection of soil samples within a location for measurements. For more discussions on experimental design, see Sections 1.4, 3.6 and 4.4, and Sit (1995).

6.5 Data Analysis

Summary statistics such as means and standard deviations may be calculated to describe the seed bank for inventory purposes. Be careful that the computation formula used corresponds to the sampling method. A common mistake is to use the formula for simple random sampling in all situations. For example, if a site is first stratified by site series or disturbance level, then the formula for stratified sampling should be used. See Cochran (1977) and Thompson (1992) for the formulae for the different types of sampling methods.

ANOVA can be used to compare the number of viable seeds among several site factors (e.g., site series, site preparation treatments, seral stages). The design of the study will determine the type of ANOVA (one-way, factorial, or split-plot) that is suitable. For this type of study, each area representing a level of the site factor of interest is the experimental unit. If several sample plots are included in each area, then these plots are subsamples only and cannot be considered replicates. If only one area is sampled for each level of the site factor, then the study will have no replication. ANOVA can still be used to compare the areas, but any differences can only be attributed to the fact that each level occupies a separate area, not to the different levels of the main site factor. The results cannot be extended to other areas with similar site factors.

Spatial analysis methods should be considered to assess the horizontal distribution of the seeds. For this objective, the data must be collected systematically. The location (x,y coordinates) and status (viable, not viable; or present, absent) of seeds at each location must be recorded. To determine whether the distribution of seeds is clumped, regular, or random, the Monte Carlo method could be used to repeatedly sample from the data set. (van der Kamp 1995).

The vertical distribution of seeds can be compared using multivariate analysis. For example, to compare seed distribution among three soil layers (e.g., litter, fermentation, and humus) between two different site series, several soil cores are randomly taken from each site. For each core, the three layers are identified and the number of viable seeds are counted. Since the depth of each layer may vary from soil core to soil core, the analysis should be based on number of seeds per soil volume. Because the numbers of viable seeds in the three layers are interdependent (a soil core with a high number of seeds in the top layer would likely have high number of seeds in the lower layers), viable seeds per volume in the two sites for the three soil layers must be compared simultaneously using multivariate analysis of variance (MANOVA). If the test is significant, then separate analysis of variance can be used to determine which soil layers differ in the mean number of viable seeds per volume for the two site types. If the MANOVA is not significant, then you could conclude that the vertical distribution of viable seeds is the same for the two site types.

For burial experiments, regression analysis may be appropriate to characterize the pattern of seed viability over time in soil. Since at each collection date, a different bag of seeds is extracted from the ground, the data collected at each date are independent, and regression is possible. If the data are recorded as percent viable seed per bag, then a transformation of the data may be necessary before regression. Transformation of data should not be done automatically for percent data; the original data should always be analyzed first. If the residual analysis indicates that the regression assumptions are strongly violated then transformation of the data could be considered. As regression is a robust technique, it is valid even if the residuals are slightly different from the normal distribution. See Section 3.7 for more discussion of regression analysis.

SECTION 7 SEED QUALITY AND VIABILITY

Though I do not believe
that a plant will spring up
where no seed has been,
I have great faith in a seed.
Convince me that you have a seed there,
and I am prepared to expect wonders.
(Henry David Thoreau)

7.1. Factors Affecting Seed Biology

7.1.1 Factors affecting dormancy and emergence

In many tree species, seed maturation is accompanied by induction of a state of dormancy. Viable seeds are considered dormant when they are placed under conditions favourable for growth, yet fail to germinate. Dormancy is a mechanism whereby species can enhance their survival by delaying germination until conditions in the external environment are conducive to active growth (Osborne 1981). The expression of dormancy is under genetic control (Naylor 1983), but is also strongly influenced by environmental factors (Steinhoff et al. 1983; Rehfeldt 1983, 1985).

Dormancy is advantageous for seeds that mature in the late summer or early fall, since seeds that germinate in the fall could be immediately exposed to harsh winter conditions. Seedling mortality likely would be high under such conditions. In nature, dormant seeds remain inactive until favourable growing conditions occur the following spring. Some seeds may remain dormant for two or more growing seasons and, depending upon the species and the environment, can remain viable for many years. The major factors affecting seed dormancy are species, seed source, crop year, and environmental factors such as temperature and moisture.

Tree seeds are generally released from dormancy only after they have been exposed to the cold, wet conditions typically found in nature during the fall and winter. Emergence occurs as temperatures rise again in the spring. Sometimes, seeds fail to germinate because water and gases cannot permeate the seed coat. Under natural conditions, such seed coat dormancy may be removed through interaction with chemicals in the soil solution. This chemical action can break down resistant seed coats or leach chemical inhibitors from the seeds. Dormancy of some seeds may be broken after the seeds pass through the digestive systems of birds or other animals, but this is not a common means of releasing dormancy in British Columbia tree species. (See Section 7.2.4 for dormancy-breaking methods). Some species, such as *Salix* and *Populus*, do not become dormant and generally germinate a short time after maturity.

7.1.2 Factors affecting germination

Moisture

Seeds naturally dehydrate as they mature. When the moisture content (mc) of a mature seed falls below 10%, the seed can survive an extended period of artificial storage (Table 7.1). (An exception is Garry oak, a member of the white oak group; the seeds exhibit recalcitrant storage behaviour and must be stored at 30% mc or more. See Section 3.4.2.) Metabolic activity is very low in seeds with less than 10% mc, so seeds must be rehydrated to physiologically active levels (usually 25% mc or more) for germination to proceed. Although dry seeds absorb water rapidly,

TABLE 7.1 *Moisture content guidelines for orthodox tree seeds (from Leadem 1996)*

Moisture content (%)	Physiological status
<5	All water is chemically bound; removal may be detrimental
5–10	Seeds may be stored for prolonged periods at low temperatures (-18°C)
<20	Seeds may revert to dormant state
25–30	Reduced risk for premature germination during stratification (2–5°C)
30–45+	Moisture level of fully imbibed seeds in preparation for stratification or sowing

physical properties of the seed coat, such as waxiness, hairiness, and thickness, may impede or restrict the entry of water into the seed.

Once seeds are fully hydrated, moisture content and respiration remain relatively constant as the essential growth processes of germination take place. The embryo grows primarily through cell division and elongation of existing cells. Cell elongation is promoted by the transport of sugars, which increases the ability of embryo cells to take up more water. As more water is absorbed by the embryo, the increased water pressure assists in the elongation of the radicle, enabling it to break through the seed coat, which by then has become softened or weakened. Water uptake continues following radicle emergence. Young germinants are very vulnerable to drought because seedling tissues are soft and unlignified; thus the availability of adequate moisture (both soil and air) is critical at this time.

Temperature

After an extensive study of more than 300 herbaceous species from a variety of habitats, plant families, and life cycle types, Baskin and Baskin (1988) concluded, "Temperature, through its influence on dormancy and germination, is the primary environmental factor regulating germination, and light and soil moisture are of secondary importance."

This conclusion undoubtedly is true for most tree seeds. Metabolic processes such as water uptake, gas diffusion, and respiration all proceed faster at higher temperatures. Germination is dependent on all these processes, and thus is strongly affected by temperature.

All seeds have an optimum temperature or temperature range for germination. For some species, the optimum temperature range is relatively narrow, while other species may be able to germinate over a wide temperature range. Dormancy-release treatments such as stratification may broaden the range of temperatures over which germination may occur (Section 7.2.4).

For most British Columbia tree seeds, the optimal temperature range for germination is between 15 and 30°C. In general, germination is considerably slower when temperatures fall below 10°C. The seeds of some hardwood species (e.g., *Acer*, *Populus*, *Salix*) can germinate at 1–5°C, but at very slow rates (Wyckoff and Zasada [1998]; Zasada et al. [1998]; Zasada and Strong [1998]). *Abies* seeds are reportedly capable of germination while buried in snow. Prolonged exposure to temperatures of 35°C or higher is usually lethal to germinating seeds.

Light

Many tree seeds (from both conifers and hardwoods) require light to germinate, although the seeds of several British Columbia conifer species appear to germinate equally well in light or darkness (Li et al. 1994). For those species with a light requirement, if all other environmental conditions have been satisfied, seeds lying on or near the soil surface will receive enough light to trigger germination. Seeds buried too deeply in the soil (more than 0.5 cm) likely would not receive enough light to germinate. As with other physiological processes, seeds must be hydrated to respond to the light stimulus. The light requirement for germination may be lessened by treatments such as stratification. For example, seeds of species that require light for germination are able to germinate in darkness once they have been stratified.

The light stimulus is received through the phytochrome system, which operates as an on/off switch for many physiological processes in plants (Figure 7.1). In light-sensitive seeds, germination is usually stimulated by exposure to red light (650–700 nm) and inhibited by exposure to far-red light (700–750 nm). Absorption of far-red light converts the pigment phytochrome$_{\text{far-red}}$ (usually the active form) back to

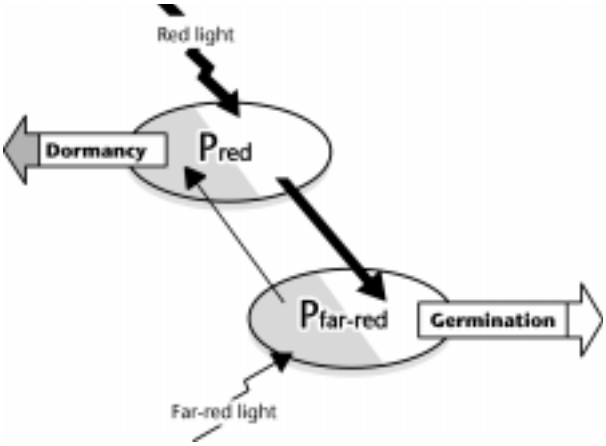


FIGURE 7.1 *Absorption of far-red light converts the pigment phytochrome$_{far\text{-}red}$ (usually the active form) back to phytochrome$_{red}$ (the inactive form) (Leadem 1996). This reaction is reversible, depending on the relative amounts of red and far-red light. In sunlight, red light is predominant, whereas far-red light is predominant in canopy-filtered light.*

phytochrome$_{red}$ (the inactive form). This reaction is reversible, depending upon the relative amounts of red or far-red light. The intensity of light required to activate the phytochrome system is low, and about 1 μW/cm^2 (comparable to bright moonlight) can be enough. The phytochrome system has ecological significance for the establishment of trees from light-sensitive seeds. Sunlight under open conditions is rich in red light, whereas light filtered through a forest canopy is predominant in far-red light. The ratio of red to far-red light also varies, depending on whether the canopy consists primarily of conifer or hardwood trees (Section 2.3.3).

Oxygen

Most seeds cannot germinate without oxygen, because oxygen is required to support the respiration that fuels the seed germination process. Oxygen requirements vary by species, but most tree seeds are able to germinate at concentrations well below the 21% by volume found in normal atmosphere; oxygen is therefore not generally considered to be a limiting factor in seed germination. In flooded soils, however, the amount of available oxygen can be limited because the air usually found in soil pore spaces has been displaced by water. The seed coat acts as a barrier to oxygen for the embryo during germination, but the coat is no longer an obstacle once the radicle emerges through the micropyle.

Other factors

The failure of a seed to germinate is not always linked to dormancy. Poor germination may be caused by seed immaturity—a problem common in high-elevation or high-latitude areas where the growing season may be shortened by adverse weather conditions. Insects, fungi, and other pests may attack seeds and severely diminish seed quality and viability (Hedlin et al. 1980; Sutherland et al. 1987; Sutherland and Glover 1991). Soil conditions, such as texture, moisture, and degree of compaction, can also affect seed germination. Soils that absorb solar radiation and hold moisture may speed the rate of germination and increase the survival of germinants (Section 8.3.1).

7.2 Seed Testing in the Laboratory

The variety and complexity of environmental factors encountered under field conditions make it difficult to assign causes for the results obtained in the field. Did an area fail to regenerate naturally because of poor seed production, predation, or seed dormancy? Or was the failure caused by environmental conditions such as drought or low temperatures during the emergence period? Often, it is not possible to separate biological from environmental factors. For this reason, it is advisable to conduct tests under more controlled conditions in the laboratory. Laboratory tests serve as controls for the tests being conducted in the field, and help identify (or at least eliminate) certain factors as causative agents.

7.2.1 Sampling methods

Detailed sampling procedures are prescribed for use by certified seed testing laboratories (Association of Official Seed Analysts 1993; International Seed Testing Association 1993). The sampling procedure begins by taking small portions, *primary samples*, at random from different positions in the seed source (e.g., seedlot). These primary samples are combined and mixed to form a single *composite sample*. This composite sample, after thorough mixing, is subdivided into a number of smaller samples (two or more) that are

taken to the laboratory for testing. These subsamples are referred to by seed analysts as the *submitted samples*. Submitted samples are often larger than required for the laboratory tests. Thus, the submitted samples are further reduced to *working samples* in the laboratory. All tests are then carried out on the working samples. Different tests require different sizes of working samples.

Seed analysts use these specialized terms to keep track of the various steps in the sampling and testing process, but the procedures are the same as those used in most research studies. For example, suppose you have to determine the quality of seeds produced by a given stand. Depending on your experimental requirements, you would collect a certain number of cones. The cones (and the seeds they contain) are the *primary samples*. All these cones and their seeds are put into a single container, and become the *composite sample*. After the seeds have been extracted from the cones, they are taken to the laboratory for testing, and become the *submitted sample*. Most likely you have collected more seeds than required for testing, so the *submitted sample* must be reduced to become the *working sample*, which for a standard germination test is four replications each of 100 seeds.

Mechanical equipment may be used (see Edwards and Wang 1995) to obtain submitted and working samples, but for most research purposes where there is a large quantity of seeds, they may be sampled by hand. To obtain the submitted sample, with the fingers and thumb kept straight and together, push your hand to the required depth in the container (Figure 7.2a). Close the fingers tightly around a portion of seeds and withdraw the hand.

In the laboratory, the submitted sample must be thoroughly mixed, then divided to give the proper working sample. The following sampling methods can be carried out using readily available, or easily constructed, equipment.

1. Spoon method Pour the seeds evenly over a tray or large sheet of paper. Using a spoon or spatula, remove small portions of seeds from several random positions until the required amount of seeds is obtained. This method is best used on small-seeded species.

2. Random cups method Pour the seeds into an ordinary dustpan so that they are evenly spread across the back edge of the pan. Place small cups or beakers (usually 8–10) randomly on a tray or large sheet of newspaper. With a single sweep of the dustpan, uniformly distribute the seeds over the containers on the tray. Whatever seeds fall into the cups become the working sample. If not enough seeds fall into the cups, return the remaining seeds to the dustpan and make a second pass. Continue until a sufficient working sample is obtained.

3. Modified halving method This is good method if you have to do a lot of sampling. Construct a grid of 6 mm (¼") or 9 mm (⅜") plywood (Figure 7.2b). Note that all cells are open at the top, but alternate cells have cardboard squares stapled to their bases. Place the grid over a tray or large sheet of paper and pour the seeds evenly over it (using the dustpan sweep as in [ii]), covering the entire grid. When the grid is lifted, some of the seeds will be retained by the cells with bases, while the rest of the seeds will remain on the tray. Repeat the process until the required amount of seeds are collected in the grid.

The description of sampling methods given above is only a brief synopsis of the topic of sampling. Mechanical methods that require specialized equipment are available for sampling large quantities of seeds. For more complete information, see Edwards (1987) or Edwards and Wang (1995).

Number of samples

Generally, four replications of 100 seeds are used for germination tests (Interna-tional Seed Testing Association 1993; Association of Official Seed Analysts 1993). However, either the number of replications or the number of seeds may be altered to suit experimental requirements. The number of seeds per replication may be reduced when few seeds are available (e.g., for germination tests of seeds from controlled crosses), but test results may be unreliable if fewer than 25 seeds per replication are used. Reducing the number of replications is not recommended; with a limited number of seeds it is better to have fewer seeds per replication and increase the number of replications. For example, if only 300 seeds are available, six replications of 50 seeds will provide better estimates of experimental error than three replications of 100 seeds.

7.2.2 Seed purity, seed weight, and moisture content

Seed purity test results report the composition (by weight) of the pure seeds of the named species, the seeds of other species, and inert matter. Seed weight is generally expressed as the weight of 1000 pure seeds, or as seeds per gram.

Seed moisture content (mc) is defined as the quantity of water lost when the sample is dried under specified conditions (see below). By international convention, the mc of seeds is expressed as a percentage of the fresh weight (FW) of the original sample. This is different from other expressions of mc for scientific purposes, which are usually made on a dry weight (DW) basis. Seed moisture content is usually calculated as:

$$\text{seed moisture content (\%)} = (M_2 - M_3) \times \frac{100}{(M_2 - M_1)},$$

where:

M_1 = weight of the empty container and cover,
M_2 = weight of the container, cover, and seeds before drying, and
M_3 = weight of the container, cover, and seeds after drying.

Determining seed moisture content is critical for long-term storage (Section 3.4), and for some dormancy

a)

1) Place open hand into the container.

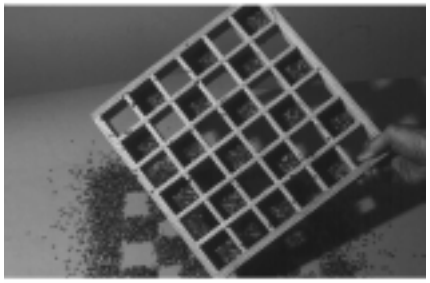
(2) Insert hand, with fingers open, deep into the container.

(3) Close fingers around the sample and withdraw the closed hand.

b)

(1) Construct a grid from plywood; alternating cells do not have a bottom.

(2) Pour seeds evenly over the grid.



(3) The working sample is retained in the grid; the remaining seeds are left in the tray.

FIGURE 7.2 *a) Sampling seeds by hand. b) Sampling seeds with a grid.*

release treatments (Section 7.2.4). The moisture content of some angiosperm seeds may be difficult to determine accurately because of their large size and the presence of volatile substances. Such seeds may have to be cut in pieces (halves or quarters) or ground before testing, and the moisture content determined by Karl-Fischer titration (Hart and Golumbic 1962) or other method.

The most commonly used method to determine DW is to dry the seeds in a forced-draught oven at 103°C ±2°C for 17h ±1 h (Association of Official Seed Analysts 1993; International Seed Testing Association 1993). First, the empty container and cover are weighed (covers and containers should have matching numbers in case they become separated). Then at least two working samples of 4–5 g each are placed in the separate containers; the containers are covered and weighed. The seeds are placed in a preheated oven, and dried in the containers with the covers removed. At the end of the drying period, the covers are replaced, and the seeds and containers are weighed again. All weights are recorded to three decimal places. In damp weather, it is recommended that containers and covers be dried, and cooled in a dessicator for at least 30–45 minutes, before they are used.

The methods for reporting seed purity, seed weight, and seed moisture content were developed primarily for use in the international seed trade to ensure standardization by approved laboratories of international seed testing associations (Association of Official Seed Analysts 1993; International Seed Testing Association 1993). Standardized methods are beneficial in that they allow easy comparison of results, and provide protocols for the expression of seed characteristics. Use of such protocols, however, does not prohibit modifying the means of reporting seed characteristics as required for research purposes.

7.2.3 Preparing seeds for testing

Determining filled seeds

Laboratory germination tests are generally conducted on filled seeds under specified conditions. For both laboratory and field studies, valid comparisons of germination cannot be made unless the total number of filled seeds used in the test is known. It is sometimes argued that it is not necessary to determine the actual number of filled and empty seeds (e.g., when assessing direct seeding trials, when testing the viability of seeds collected from seed traps, or when using the data to calculate the regeneration potential based on the seeds available on the site). However, in all cases, the number of filled seeds is of critical importance. How, for example, do you interpret a reported germination of 50%? You must know the number of filled seeds to respond. If the seeds are 100% filled, then germination is only 50%, but if only 50% of the seeds are filled, then germination is 100%.

To ensure that only filled seeds are used in the test, seeds may be X-rayed before testing, and the empty seeds removed. Otherwise, ungerminated seeds remaining at the end of the test must be examined to determine whether the seeds are filled or empty. The seeds may be cut open, or they may be dried and X-rayed (see Section 7.2.6 for details). Tetrazolium chloride can be used to assess the viability of different tissues of ungerminated filled seeds (Section 7.2.6).

Surface sterilization

In some instances, mould can significantly reduce germination of infected seeds, especially if the seeds are poor quality. Surface sterilization of seeds with hydrogen peroxide may be beneficial, but sterilization treatment should be considered the exception rather than the rule. Germinating seeds are extremely sensitive to phytotoxic substances, so preliminary testing is essential. Tests should be done on a small sample of dry seeds. If germination is improved by the treatment, sterilize dry seeds by immersing them in 3% hydrogen peroxide (H_2O_2) for 5 minutes, followed by three rinses with de-ionized water.

It is possible to quantify the degree of mould infestation (or other characteristic) by grouping the seeds into classes according to predetermined criteria (see Table 7.2). Assessing the seeds for mould at the end of 1 week and again at the end of the test is usually enough to obtain meaningful data. Such data can be analyzed using nonparametric methods (Sections 5.5 and 8.3.4).

Hydration of seeds

Before beginning the germination test, seeds of most British Columbia conifer species are stratified. Table 7.3 (conifers) and Table 7.4 (hardwoods) present treatments currently used in British Columbia.

Seeds must be physiologically active to respond to treatment (Section 7.1), so most conifer seeds are soaked in water for 24 hours at room temperature (20–25°C) before stratification or testing. Notable exceptions are *Abies* seeds, which are soaked for 48 hours, and western redcedar seeds, which are not soaked before germination tests. For soaking, seeds should be covered with a volume of de-ionized water (or tap water if it is of reasonable quality) equal to at least twice the volume of the seeds.

TABLE 7.2 *Classification of mould infestation in seeds*

Class	Infected seeds (%)
1	0
2	1–25
3	26–50
4	51–75
5	76–100

Each replication should be labelled with the appropriate code for species, seedlot, treatment, and replication number. (Four replications of 100 seeds are generally used. See discussion above.) Seeds that do not require stratification may be surface-dried on paper towels and incubated immediately after soaking. Seeds of species that require chilling are put in capped plastic vials or plastic bags for stratification at 2–5°C, according to the recommendations for the species (Tables 7.3 and 7.4). The volume of the stratification container should be much larger than that of the seeds to allow ample space for air above the seeds. If cold storage area permits, it is sometimes convenient to stratify seeds in the containers that will be used for incubation.

To schedule the work load to coincide with a standard work week, it is convenient to begin soaking seeds on Monday (48-hour soak) or Tuesday (24-hour soak) to start the experimental incubation on Wednesday. Since tests are conducted normally for a specified number of weeks (usually 3–4) (see Table 7.3), tests that begin on Wednesday will end

TABLE 7.3 *Stratification and incubation regimes for British Columbia conifer seeds(Leadem 1996)*

Species name	Treatment		Incubation	
	Soak	Stratification	Temp (°C)[a]	Length (days)
Abies amabilis	48 hours [b]	4+12 weeks [c]	25/15	28
Abies grandis	48 hours [b]	4+12 weeks [c]	25/15	28
Abies lasiocarpa	48 hours [b]	4+12 weeks [c]	25/15	28
Chamaecyparis nootkatensis	10–28 days [d]	16–20 weeks	30/20	21
Picea glauca	24 hours	3 weeks	30/20	21
Picea sitchensis	24 hours	3 weeks	30/20	21
Pinus contorta	24 hours	3 weeks	30/20	21
Pinus monticola	48 hours [b]	4W+8C [e]	30/20	21
Pinus ponderosa	24 hours	3 weeks	30/20	21
Pseudotsuga menziesii	24 hours	3 weeks	30/20	21
Thuja plicata	none [f]	none	30/20	21
Tsuga heterophylla	24 hours	4 weeks	20°C	28
Tsuga mertensiana	24 hours	4 weeks	20°C	28

W = warm stratification; C = cold stratification.

[a] The first temperature is given during the 8-hour light period, the second temperature is given during the 16-hour dark period. If only one temperature is shown, light is given for 8 hours, but the temperature does not alternate.

[b] Seeds soaked for 48 hours receive a water change after 24 hours.

[c] Stratification-redry treatment: seeds are soaked for 48 hours, drained to remove excess water, and placed, with no surface drying, at 2–5°C for 4 weeks. After stratification for 4 weeks, the seeds are dried to 30% moisture content, then chilled for an additional 12 weeks.

[d] Water should be changed every second day.

[e] Warm/cold stratification: seeds are soaked, drained and kept at 20–25°C for 4 weeks, then stratified for 8 weeks at 2–5°C.

[f] Seeds should be heavily misted for several days after sowing.

on Wednesday, allowing 2 days of the work week for cleanup or other tests.

7.2.4 Dormancy-breaking procedures

Dormant seeds can be stimulated to germinate using treatments that emulate natural conditions or satisfy certain physiological requirements (Taylorson and Hendricks 1977). The choice of a suitable dormancy-release treatment can increase germination rates, and broaden the range of environmental conditions under which germination can occur.

Stratification

Stratification is the most consistently effective dormancy-release treatment for British Columbia tree seeds. Stratification enables seeds to germinate more quickly and completely, and can sometimes eliminate the need for other special conditions, such as light or the close control of temperatures. Even when the final germination percentage remains the same, the germination rate of most tree seeds is often improved by stratification, especially when seeds are incubated at low temperatures (Figure 7.3) (Table 7.5).

TABLE 7.4 *Stratification and incubation conditions for British Columbia hardwood seeds*

Species name	Treatment		Incubation	
	Soak	Stratification	Temp (°C)[a]	Length (days)
Acer macrophyllum	48 hours	45–130 days	20	28
Alnus rubra	24 hours	14–28 days [b]	30/20	21
Arbutus menziesii	24 hours	60 days [c]	–	–
Betula	none	none	30/20	21
Betula papyrifera var. *neoalaskana*	none	none	30/20	21
Cornus nuttallii	24 hours	90 days [c]	–	–
Fraxinus latifolia	24 hours	2W+7C [d]	30/20	56[e]
Malus fusca	48 hours	90 days [c]	–	–
Populus balsamifera ssp. *balsamifera*	none	none	30/20	10
Populus balsamifera ssp. *trichocarpa*	none	none	30/20	10
Populus tremuloides	none	none	30/20	10
Prunus emarginata	48 hours	90–126 days [c]	–	–
Quercus garryana	48 hours	none	20	28
Rhamnus purshiana	none	none	–	–
Salix amygdaloides	none	none	–	–
Salix bebbiana	none	none	–	–
Salix discolor	none	none	–	–
Salix exigua	none	none	–	–
Salix lucida ssp. *lasiandra*	none	none	–	–
Salix scouleriana	none	none	–	–
Salix spp.	–	–	30/20	14

W = warm stratification; C = cold stratification.
[a] The first temperature is given during the 8-hour light period, the second temperature is given during the 16-hour dark period. If only one temperature is shown, light is given for 8 hours, but the temperature does not alternate.
[b] Ager et al. (1994).
[c] Schopmeyer (technical coordinator, 1974).
[d] Warm/cold stratification: seeds are soaked, drained, and kept at 20°C for 2 months, then stratified for 7 months at 2–5°C (Schopmeyer, technical coordinator, 1974).
[e] International Seed Testing Association (1993).
– no information available.

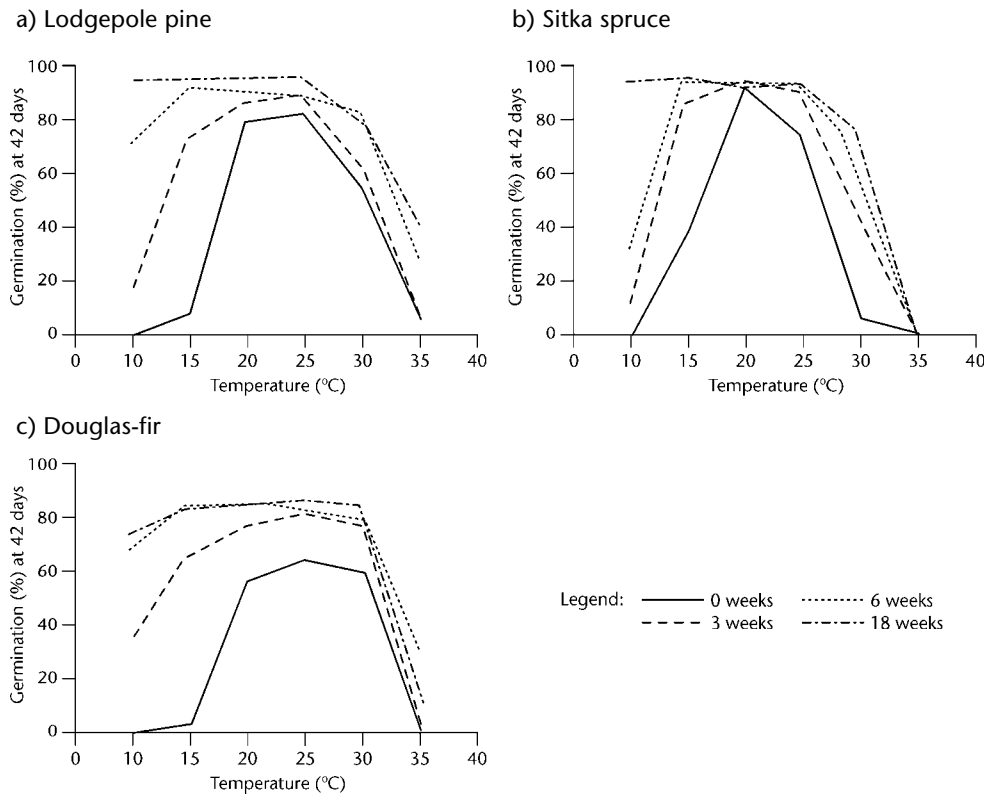


FIGURE 7.3 *Germination of (a) lodgepole pine, (b) Sitka spruce, and (c) Douglas-fir at different temperatures and after stratification for 0, 3, 6, and 18 weeks (Leadem 1996; data from Jones and Gosling 1994).*

TABLE 7.5 *Summary of stratification methods: (a) Conventional stratification; (b) Stratification-redry; (c) Stratification (bigleaf maple); (d) Stratification (warm + cold); (e) Stratification (hardwoods)*

(a) Conventional stratification

Process	**Soak**	**Stratify**	**Incubate**
Time	24 or 48 h	3–4 weeks	3–4 weeks
Temperature	20–25°C	2–5°C	20–25°C
mc	to >30%	30–60%	30–60%

Stratification simulates winter conditions by exposing moist seeds to cold temperatures. Seeds are soaked in water (hydrated) usually for 24 hours, drained, then placed in a plastic bag or other container, and refrigerated (2–5°C) for several weeks. With the exception of western redcedar seeds (not considered to be dormant), all British Columbia conifer seeds require stratification for best germination (Table 7.3). Although *Salix* and *Populus* seeds are not stratified, many other British Columbia hardwoods (e.g., *Acer*, *Arbutus*, *Cornus*, *Fraxinus*) have a chilling requirement (Table 7.4). Even hardwood seeds that can germinate without stratification (e.g., red alder) often benefit from chilling when they are incubated under low temperatures (Ager et al. 1994). Damaged seeds, or those of low vigour, may deteriorate during stratification (Leadem 1986); in such cases, the seeds should be sown without chilling.

TABLE 7.5 *(b) Stratification-redry*

Process	Soak	Stratify	Redry	Stratify	Incubate
Time	48 hours; change after 24 hours	4 weeks	4–8 hours	12 weeks	3–4 weeks
Temperature	20–25°C	2–5°C	20–25°C	2–5°C	20–25°C
mc	to >30%	30–60%	to 25%	25%	25–60%

Conventional stratification techniques may be in-sufficient to stimulate germination in some conifer species. The true firs (amabilis, grand, and subalpine) respond best to a two-part stratification called stratification-redry (Edwards 1985; Leadem 1986, 1989). Seeds are hydrated for 48 hours and then stratified for 4 weeks at 2–5°C; seed moisture content is high, usually above 40%. The seeds are then dried to 25–30% mc and chilled for an additional 12 weeks. At such low moisture contents, seeds receive the extended chilling they require, but moisture levels are too low to permit emergence of the radicle (i.e., evident germination). The stratification-redry treatment has also been found effective for some sources of Douglas-fir seeds, but not for other tree species.

TABLE 7.5 *(c) Stratification for variable dormancy (hardwoods)*

Process	Soak	Stratify	Incubate
Time	48 hours; change after 24 hours	X weeks (to 5% germ.) X = 60–120 days	3–4 weeks
Temperature	20–25°C	2–5°C	20–25°C
mc	to >30%	30–60%	30–60%

Some species such as bigleaf maple require extended stratification, but the optimum duration for individual seed sources is unknown. An empirical procedure for seeds with variable dormancy is to place the hydrated seeds (> 30% mc) at 2–5°C, and maintain them at low temperatures until about 5% of the seeds germinate. For bigleaf maple, this is about 60–120 days (J. Zasada, pers. comm., 1996). Germination of the least dormant individuals at low temperatures generally indicates that dormancy also has been released in the remaining, more dormant seeds, and that the seeds may be transferred to warmer temperatures for testing or seedling production.

TABLE 7.5 *(d) Stratification (warm + cold)*

Process	Soak	Stratify-warm	Stratify-cold	Incubate
Time	48 hours; change after 24 hours	X weeks (to 5% germ.); X = 3–5 weeks	6 weeks	3–4 weeks
Temperature	20–25°C	20–25°C	2–5°C	20–25°C
mc	to >30%	30–60%	30–60%	30–60%

A variation of stratification (c) is the dormancy treatment used for western white pine. The usual recommendation for releasing dormancy in western white pine is 30 days warm stratification (20–25°C) followed by 60 days cold stratification (2–5°C). However, the duration of warm stratification varies, depending upon the seed source. Seeds are kept under warm conditions until about 5% of the seeds show evidence of germination, then the seeds are immediately transferred to cold temperatures (D.W.G. Edwards, pers. comm., 1996).

TABLE 7.5 *(e) Stratification for variable dormancy in deeply dormant hardwoods*

Process	Soak	Stratify	Incubate
Time	Until mc = Y	X+2 weeks (to 10% germ.); X = 1–8 months	3–4 weeks
Temperature	20–25°C	2–5°C	20–25°C **or**
mc	(e.g., Y = 30%)	Y% mc	store at 2–5°C, 10% mc

A procedure developed for beechnuts and other European hardwood seeds by Suszka (1974) may also prove effective for removing the dormancy of British Columbia hardwood seeds. It is similar to the variable-dormancy treatment described above, but also involves hydrating the seeds to a predetermined moisture content (Y), depending on the species (e.g., 30% mc for beechnuts), then maintaining the seeds at this mc for X+2 weeks at 2–5°C. The duration of treatment, represented as X (in weeks), is the time when 10% of the seeds have germinated. This length of stratification is enough to break dormancy, but because seeds are chilled at reduced moisture levels, germination is prevented. After treatment, the seeds can either be sown, or dried to below 10% mc and stored for several years (Muller and Bonnet-Masimbert 1989; Muller et al. 1990). Such dormancy-breaking treatments are usually applied after storage, but they can also be applied before storage (i.e., immediately after collection). The time period X can be considered as an indication of the degree of seed dormancy, and potentially could be used to compare the dormancy levels of different seed sources. The period X is 1–3 months for beechnuts (*Fagus sylvatica*), 5–6 months for wild cherries (*Prunus sativum*), and 6–8 months for ash (*Fraxinus* spp.) (Muller 1993; Suszka et al. 1996).

Other stratification treatments

Subalpine larch seeds and other species that are difficult to germinate may be stratified *in situ* using a procedure that more closely approximates natural conditions under the snowpack. To stratify subalpine larch, Carlson (1994) used plastic tubes (3.8 cm diameter 14 cm long) which were filled to within 2 cm of the top with a peat-perlite mix, then thoroughly wetted. Seeds were covered with about 2 mm of soil, firmly pressed, and covered with 2 mm of fine gravel to prevent soil from splashing during irrigation. The trays of tubes were thoroughly watered, covered with clear polyethylene to retain moisture, and placed in a cold room at 2°C for 30 days. After 30 days, the tubes were removed from cold storage and placed under conditions suitable for germination.

Yellow-cedar seeds can be difficult to hydrate and require exceptionally long stratification. The seeds are soaked for 10 days at room temperature (20–25°C), then stratified (2–5°C) for 4 months. Even with long stratification, the seeds often germinate poorly. It may be necessary to pregerminate the seeds by putting them on damp peat in shallow dishes, covering the dishes with plastic wrap, and incubating them at constant 20°C. As the seeds germinate, they can be pricked out into nursery containers.

Light treatments

A number of tree species require light to release seeds from dormancy (Section 7.1.2). Experimentally, light treatment may be given in the form of a single exposure prior to incubation, or as daily exposures throughout the incubation period. Studies involving the responses of seeds to light require suitable sources of red and far-red radiation. Filters must restrict the wavelengths to within a narrow band, and provisions must be made to reduce heat levels around the seeds if exposure times are extended. A transparent water bath at least 5 cm deep is an inexpensive and effective way to absorb excess infrared radiation.

Before using any filter system, the transmission spectrum should be determined with a spectrophotometer. Some sources for red/far-red light include the following:

- Light sources can be constructed using Corning Plexiglas filters (red light 650 nm; far-red light 750 nm) with two 15W cool white fluorescent tubes for the white and red light treatments and one 75W incandescent bulb for the far-red treatment (Haeussler and Tappeiner 1993).
- An inexpensive red/far-red light source may be made from a desk lamp and cellophane paper (from art supply store); the red light (transmission range: 590–700 nm) provided by a red or white incandescent bulb (40W) or a cool white fluorescent tube filtered through two layers of red cellophane or red Plexiglas (Witham et al. 1971); the far-red light (transmission range: 700–735 nm) provided by a 40W or 60W incandescent bulb filtered through four layers of blue, one green, and two layers of red cellophane. High wattage bulbs are not recommended because they generate too much heat, and may bleach the pigments in the cellophane with prolonged exposure. With low-wattage bulbs, the filter may be placed in front of the light source and tightly taped to the lampshade. An alternative method is to place the sample in a light-tight box with the cover removed and replaced by the filter.

Energy levels from most light sources tend to be higher in the red region than in the far-red portion of the spectrum. Haeussler and Tappeiner (1993) compensated for the lower energy levels in the far-red region by exposing the seeds to red light for 5 minutes and extending exposure to the far-red light source to 10 minutes. Light energy levels of red and far-red sources do not have to be exactly equivalent. *Betula papyrifera* seeds demonstrated definite red/far-red responses to red (600–700 nm) and far-red (700–850 nm) light sources which measured 21 W m^{-2} and 7.3 W m^{-2}, respectively (Bevington 1986).

Conditions for dark (controls) can be established by wrapping germination dishes (or other containers) in aluminum foil or other opaque covering. The dishes should only be examined in a dark room under the illumination of a dim green safelight. Use of a green incandescent bulb is not recommended, as such light sources can emit in the red and far-red regions. A safelight can be constructed of two 15W cool white fluorescent lamps covered with 18 layers of green cellophane. Radiation from this light source did not induce germination when used to count *Betula papyrifera* seeds (Bevington 1986). A green safelight can also be made from a flashlight by covering the lens with an appropriate filter.

Other dormancy-release treatments

Other dormancy treatments (Table 7.6) will be only briefly described here because they are not widely used for temperate tree species. Scarification is an important technique for breaking the dormancy of many hard-seeded legume tree species that are an important component of tropical forests. Hard seed coats are a means of protecting seeds from fungal and insect attack under conditions of high temperatures and high humidity. Mechanical or chemical degradation of the seed coat is necessary for germination, and in nature is often facilitated by seeds passing through the intestinal tract of birds and other animals.

Few temperate forest species require scarification —Schopmeyer (technical coordinator, 1974) does not recommend it for any British Columbia trees—but seed coats of pines are sometimes clipped to facilitate germination (Hoff and Steinhoff 1986; Leadem 1986).

Application of plant growth regulators (especially gibberellic acid and cytokinin) have been shown to enhance germination of angiosperms, but have limited effectiveness for conifer species (Leadem 1987).

7.2.5 Laboratory germination tests

Standard germination tests are widely used because they provide consistent results and allow comparisons of tests conducted in different laboratories. For field tests, however, the relatively favourable conditions of standard tests may give an overly optimistic view of germination. Valuable data can be obtained by conducting germination tests under conditions similar to those found in study sites. Temperatures used should range from suboptimal to optimal (and possibly beyond) to gain a fuller appreciation of species' response. Conducting tests under the relatively cool temperatures that mimic field conditions may require more time, but will be more relevant to understanding responses in field studies.

The measures of germination used for comparing laboratory and field results are also important. Since, in natural conditions, the speed and time of emergence can be critical (e.g., in areas where moisture is readily available only in early spring), measures such as germination rate and speed may be the best indicators of field performance. Laboratory tests, by eliminating some of the variability associated with field tests, can assist in interpreting field data so that the factors affecting germinant establishment and survival may be more clearly identified.

TABLE 7.6 *Dormancy release treatments for tree seeds (Leadem 1996).*

Treatment	Description
Stratification	Moist chilling at 2–5°C; removes metabolic blocks, weakens seed coats, increases germination promoter levels
Light	Exposure to specific wavelengths; stimulates the phytochrome system
Leaching	Soaking in water; removes inhibitors from seed coats
Scarification	Chemical (sulphuric acid) or mechanical (abrasion) treatment: breaks down seed coats
Plant growth regulators (hormones)	Enhances natural levels in favour of germination regulators
High oxygen	Supplies respiration; removes metabolic blocks concentrations

Incubation of seeds

The temperature and photoperiod conditions for incubation will be determined by the experimental requirements. Standard incubation conditions commonly used for British Columbia tree seeds are 30°C for 8 hours (with light) and 20°C for 16 hours (dark). The true firs (*Abies* spp.) generally respond better to 25°C for 8 hours and 15°C for 16 hours (Leadem 1989), with light being given during the high-temperature period.

The seeds may be incubated in 12 × 12 cm plastic boxes, filled with a medium consisting of two layers of filter paper (such as Whatman #1) covering one layer of Kimpak™ (a multilayered crêped wadding). For this size of container, 50 mL of de-ionized water is added to moisten the media; this amount is sufficient for a germination test of 3–4 weeks duration (provided the lids fit tightly and there are no cracks in the boxes). The seeds are spread evenly on the moist filter paper so that no two seeds are touching and all are in direct contact with the media, then the lids are replaced on the boxes. Petri dishes filled with several layers of filter paper may also be used, but Petri dishes have a smaller surface area for testing, and the medium tends to dry out more quickly.

Test environments are fairly consistent over the incubation period since most laboratory tests are conducted in covered dishes incubated in controlled environment chambers. However, the dishes should be randomly arranged on shelves in the chamber to guard against the introduction of systematic error due to consistent differences in light intensity, temperature, or other factors. Random label lists can be generated from the codes used to identify seed source, treatment, replication number, or other experimental variables.

Germination criteria

The criteria for germination may be the cracking of the seed coat, or development of all structures necessary for a normal seedling (i.e., root, stem, cotyledons). For many studies, seeds are considered to have germinated once the length of the radicle is four times the length of the seed coat. Use of a radicle length shorter than four times is not recommended because of the greater probability of counting abnormal ("stunted") germinants as "normal." For *Abies* species, which have relatively large seeds, a radicle that is twice the length of the seed coat may be satisfactorily used as a germination criterion. Typically, for research studies, germinants are counted 3 times a week for the duration of the test, which is usually 3–4 weeks in a standard laboratory test (Tables 7.3 and 7.4). During peak germination periods, it is advisable to count daily. Many service laboratories count germinants once a week, which is too infrequent for research studies that require precise information on when and how changes in germination occur.

Under field conditions or in the nursery, the term *emergence* is generally used instead of *germination*. In epigeal germination (Figure 7.4b), the standard criterion for emergence is the time when the seed coat has lifted off the soil surface or, in hypogeal germination (Figure 7.4a), when the epicotyl is clearly evident.

Salix and *Populus* germinants (Figure 7.5) exhibit a departure from the usual pattern of epigeal germination (Simak 1980). Willow and poplar seeds contain only a large embryo, surrounded by a transparent coat. Within the first day of germination, the hypocotyl increases in length and a ring of fine hypocotyl hairs (the coronet) arises and attaches the seedling to the substrate. Thereafter, the elongating hypocotyl rises into a vertical position and the radicle starts to elongate.

For nursery or field tests, test duration is typically 6–8 weeks, but may be longer depending upon environmental conditions. See Section 7.3 for information on field germination tests.

It is best to write entries on data sheets when counting germinants, rather than entering data directly into a datalogger or computer, since it is easy to make errors during the counting process. The first day that visible signs of germination activity (chitting) occur should be noted on the data sheets, as this will be later used to indicate the initiation of the germination curve. The number of germinated seeds is

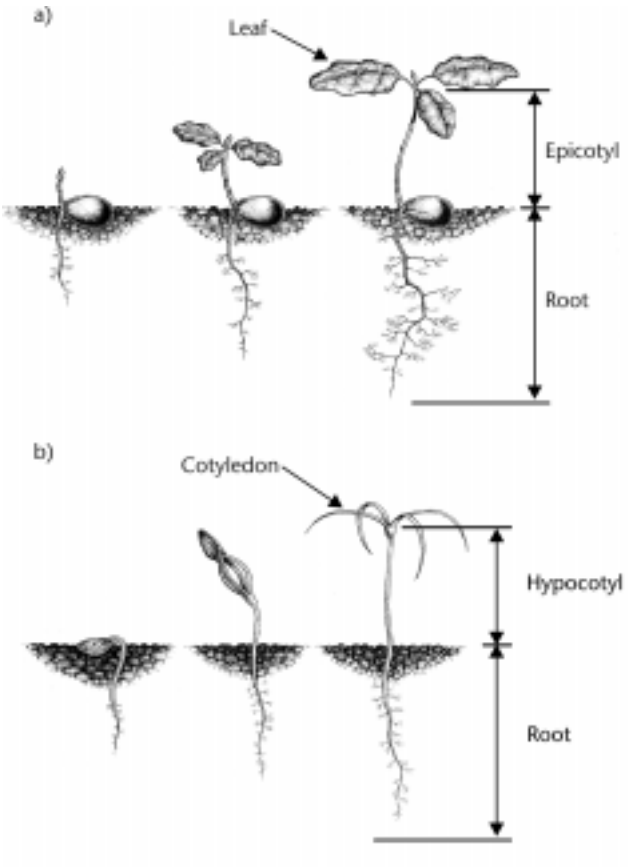


FIGURE 7.4 *Stages of germinant development in hypogeal and epigeal germination (Leadem 1996): (a) Garry oak, an angiosperm, illustrates hypogeal germination, in which cotyledons remain below the ground; (b) white spruce, a gymnosperm, exhibits epigeal germination, in which cotyledons are raised above the ground.*

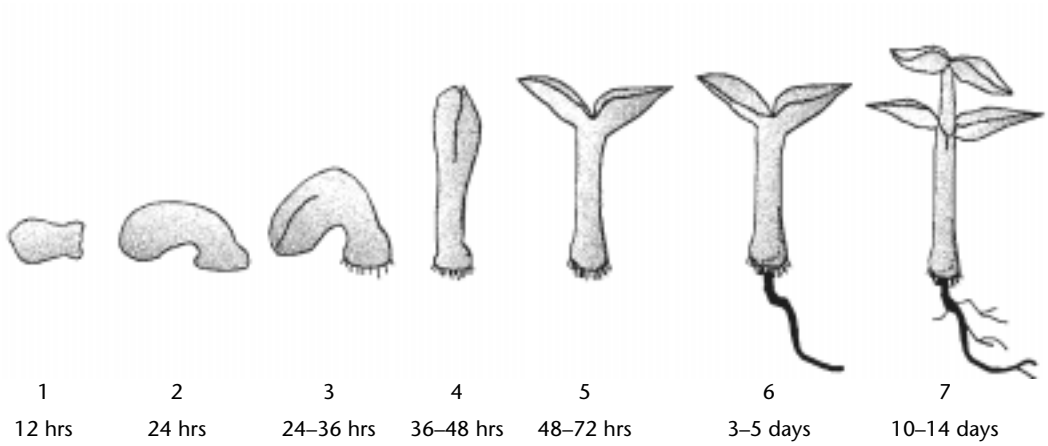


FIGURE 7.5 *Stages of germination for* Populus *seeds, showing time period in which each stage usually occurs (Wyckoff and Zasada [1998]). Within the first day of germination, the hypocotyl increases in length and shows a positive geotropic bending (Stages 1, 2). Hypocotyl hairs (the coronet) arise and attach the seedling to the substrate (Stage 3). Then, the elongating hypocotyl rises into a vertical position, and the radicle emerges (Stage 4). The seed coat bursts and is removed as the hypocotyl elongates and the cotyledons expand (Stages 5, 6). Epicotyl development begins after about 10–14 days (Stage 7).*

recorded in columns under the date on which the counts are made. If large numbers of abnormal germinants are observed, a classification system (Clark 1994) may be used to record the nature of the abnormality. On the last day of the test, the number of germinants is recorded, as well as the number of ungerminated seeds. The reason(s) for lack of germination should be noted, if known. If seeds have not been X-rayed before testing and a precise result is required, cutting tests (Section 7.2.6) should be done on all ungerminated seeds to determine the exact number of filled seeds in the test.

Additional information on methods and procedures for testing tree seeds of Canadian conifer species can be found in Edwards (1987).

Germination measures

Germination percentage, the most common expression of seed germination, is calculated as:

$$\frac{\text{number of germinated seeds}}{\text{number of filled seeds}} \times 100\%$$

The estimated variance, although not a germination measure *per se*, is a valuable aid for interpreting germination data. In some instances, the estimated variance may be as important as the mean germination percentage. Consider a study to examine the effects of different seed sources on germination. If the resulting data have large variance, then the results must be narrowly interpreted concerning seed origin. On the other hand, a data set with small variance would suggest a more generalized species response; it may also suggest that factors other than seed source should be examined for their impact on germination. The estimated variance may be used to demonstrate the benefits of certain treatments; the germination of stratified seeds, for example, is far less variable than that of unstratified seeds.

Germination rate has both practical and ecological significance. The rate at which seeds germinate has long been recognized as an element of seed vigour, and an indication of the ability of a germinant to become successfully established as a seedling (Leadem 1988). Many seed treatments increase the rate of germination, but not the total germination. In natural regeneration studies, germination rate may be more important than total germination, particularly for sites where moisture and plant competition are limiting factors. Germination rate is often expressed as R_{50}, or the number of days it takes 50% of the sown seeds to germinate. A similar term, $R_{50'}$, or *germination speed*, is the number of days for 50% of the germinating seeds to germinate. Differences in germination rates also can be presented graphically using nonlinear regression analysis procedures (Tipton 1984) (see Section 7.5).

Germination value (GV) (Czabator 1962), an expression that combines the speed and completeness of germination into a single number, is calculated as:

GV = peak value (PV) × mean daily germination (MDG),

where:

PV = the maximum quotient obtained by dividing the number of accumulated daily germination by the corresponding number of days, and

MDG = total germination divided by the number of days in the test.

The absolute magnitude of GV depends upon the species, but values usually range from 10 to 60 (Table 7.7). Because of the germination characteristics of different species, a GV of 20 means something different for pine than for fir or for hemlock. Pines tend to have relatively high GV values (i.e., more than 50) because they germinate very quickly, while the GV of the true firs, which germinate slowly, is usually low (about 10–15). Germination values of spruces generally are intermediate (35–40). Germination values have no units, and have not been widely accepted by those who prefer separate reporting of germination rates and total germination.

TABLE 7.7 *Germination values for British Columbia conifers*

Species	Germination value (GV)
Pacific silver fir	10
grand fir	12
subalpine fir	13
Douglas-fir, coastal	40
Douglas-fir, interior	52
western hemlock	19
western larch	52
lodgepole pine, coastal	52
lodgepole pine, interior	58
western white pine	18
yellow (ponderosa) pine	45
Sitka spruce	35
interior spruce	40
Sitka/interior hybrids	36
western redcedar	25
yellow-cedar	5

Source: D. Kolotelo, B.C. Ministry of Forests, Tree Seed Centre, Surrey, B.C. from results of germination tests conducted from 1990 to 1995.

7.2.6. Quick tests and other viability tests

A standard germination test requires a minimum of 3 weeks to complete; with stratification, the test can take 6 weeks or longer. Sometimes standard tests cannot be done because of time constraints or the lack of adequate facilities. In such cases quick tests of seed viability can provide reasonably good estimates of seed quality. The quick tests most commonly used for tree seeds are the hydrogen peroxide, tetrazolium, X-ray, and cutting and excised embryo tests. No single quick test is best in all situations, and each has its advantages.

The basic principle behind all quick tests is that the seeds are treated by some "quick" procedure, then classified according to established criteria. Quick test results of several samples can be calibrated by correlating the percentage of germinable seeds determined by quick test to the percentage of seeds that germinate in a standard test. Quick tests may take less time than standard germination tests, but they are usually more labour intensive, and the results can be more variable than results from standard germination tests.

For further information about quick tests and other tree seed testing methods, see Leadem (1984), Edwards (1987), Association of Official Seed Analysts (1993), International Seed Testing Association (1993), and Edwards and Wang (1995). The specific procedures used in the B.C. Ministry of Forests Tree Seed Physiology Laboratory are given in Clark (1994).

Hydrogen peroxide test

Of the three tests, the hydrogen peroxide method is the only one that actually measures growth. The primary advantages are objectivity and simplicity, and since this test requires less time and less equipment than most other viability tests, it is also the least expensive.

The hydrogen peroxide test requires 7 days to complete and can be difficult to do on small seeds. It is carried out by cutting 1–2 mm off the micropylar end of the seed coat, and incubating the seeds in a

1% hydrogen peroxide (H_2O_2) solution at 20°C. The test is ended after 1 week, and seeds with radicles longer than 1 mm are counted as viable.

Tetrazolium chloride test

The tetrazolium chloride (TZ) test focuses directly on the physical and physiological condition of the embryo and endosperm (or, in conifers, the megagametophyte). Evaluation is based on the degree and location of stained and unstained areas. Because results can be obtained within 8–24 hours, tetrazolium is faster than all quick tests except the X-ray method. However, the interpretation of staining patterns relies heavily on the expertise of the analyst.

To conduct the test, seeds are soaked in water overnight to soften the coats, then a thin longitudinal slice is cut through the coat and storage tissue to hasten penetration of the tetrazolium (2,3,5-triphenyl tetrazolium) solution (Figure 7.6). The seeds are soaked in a 1% TZ solution at 38°C for 2–8 hours. The time required for adequate staining varies with the species and seed source; excessive incubation should be avoided since dark staining may mask weakened tissues. The intensity and location of the stain is an indication of whether the embryo is vigorous enough to produce a viable seedling. The uniformity of staining and the rate at which staining develops in different areas may indicate weakened tissue. Necrotic, unstained areas generally are of greater significance than stained areas, especially if they occur in the shoot or radicle meristems. In the radicle area, the meristem is located just behind the radicle tip, while in the shoot, meristematic growth emanates from the base of the cotyledons. Meristematic areas must be well stained to produce a viable seedling.

For additional information, refer to Moore (1976), Leadem (1984), and International Seed Testing Association (1993).

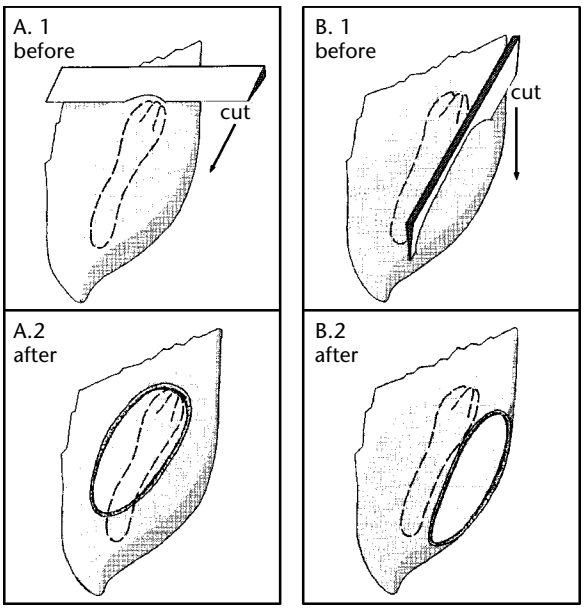


FIGURE 7.6 *Cutting diagram for the tetrazolium test. A thin longitudinal slice is cut through the seed coat and storage tissue to hasten penetration of the tetrazolium solution. Cuts may be made horizontally to the long axis of the seed (1a, b), or perpendicular to the long axis (2a, b).*

X-ray test

The X-ray method is the most rapid of the quick tests. Only a few minutes are required to produce an X-ray image, and a large number of seeds may be examined in a short time. Soft (low-energy) X-radiation does not affect seed germination or cause any apparent chromosome damage (Kamra and Simak 1965). Viability evaluation is based on a physical examination of seed contents, and results have been shown to correlate well with germination test results (Leadem 1981). The primary drawback is the high cost of X-ray exposure and developing equipment; but once equipment is acquired, the cost of materials compares favourably with most standard testing methods.

Celluloid film or photographic paper may be used for X-ray exposures. Kodak Type M industrial X-ray film provides good resolution and a permanent record, but requires a darkroom and developing apparatus. Polaroid film can be used for making seed radiographs (Edwards 1973); a darkroom is not required but the film is more expensive than X-ray film or paper. Photographic paper is less expensive than X-ray film and can be processed in an instant processor, but the images have relatively poor resolution and are not permanent unless special provisions are made. Photographic paper is normally used when a permanent record is not required.

A monitor can be attached to the X-ray machine and the images viewed directly. The advantage of such a system is that no developing is required; the

disadvantages are the lack of a permanent record, and the much higher cost of equipment.

Seeds can be placed directly on the X-ray film or photographic paper, but for most routine work a holder is used. Holders may be clear plastic boxes or compartmentalized trays, but any material intended for use as a seed holder should first be tested for X-ray penetrability. The seeds and film or paper are placed in the shielded cabinet of the X-ray machine, and the exposure is made. Typical settings for a Faxitron (brand name) X-ray machine are 3 mA and 15 kV with an exposure time of 2 minutes. The film or paper is developed, and each seed image is examined. Seed development and viability are determined by evaluating the density, shape, and location of opaque matter (bright areas) in the X-rays. If the purpose is to determine the presence of filled and empty seeds, then the seeds in the holder must be compared to the corresponding X-ray images of the same seeds. Empty seeds may be removed using forceps or a vacuum pencil (the type used for manipulating electronics components). Care must be taken not to disturb the seeds in the holder during the X-ray process.

X-rays are best performed on dry seeds (<10% mc) because moisture (which appears opaque and bright in X-rays) tends to obliterate the details of seed contents. X-rays may still be used on moist seeds instead of a cutting test, or to determine the number of ungerminated filled seeds left at the end of a germination test. Simply dry the remaining seeds at room temperature (20–25°C) for 6–8 hours (or overnight), then X-ray as usual.

Cutting tests

The cutting test is done by cutting a longitudinal section through the seed to expose the embryo and storage tissue. Although not as reliable as viability tests, cutting tests are often used in field situations to assess seed development and the presence of insects or disease.

Take 10–20 seeds at random and examine them by slicing each seed into equal parts with a razor blade or scalpel. Unless the seed has been exactly bisected, you cannot observe the exact extent of embryo elongation. The contents of each seed are more accurately appraised if a 10-power hand lens is used. The preferred method for slicing a seed is to stand it on its narrower edge on a cutting surface, using forceps to hold it in place. A vertical cut is then made downward through the seed between the prongs of the forceps. Another method is to place the seed flat on a firm surface, and to slice it parallel to the cutting surface (i.e., horizontally). A third, but less desirable method, is easier to perform under field conditions, and is suitable for small seeds such as spruce, lodgepole pine, western redcedar, and yellow-cedar: lay the seed flat on the cutting surface and make a vertical cut downwards. Since the seed is cut through its narrowest dimension, it may be more difficult to accurately assess storage tissue development. See Eremko et al. (1989) and Kolotelo (1997) for diagrams and additional information.

Excised-embryo tests

The excised embryo test is generally less used than other quick tests because it is more labour intensive, and interpretation may be more difficult. Seeds for the excised embryo test are soaked in tap water for 24–96 hours. Species that require mechanical or chemical scarification must first be given the appropriate treatment. Embryos are excised from soaked seeds with the aid of a scalpel or razor blade, then incubated on moistened filter paper for 2–14 days at 20–25°C with 8 hours of light per day. Evaluation is based on chlorophyll development and growth of the embryo. The test is effective for hardwood species because the seeds turn green quite readily, and is a recommended procedure for *Fraxinus* spp. and *Malus* spp. (International Seed Testing Association 1993). Evaluation in conifers can be more difficult because embryo extension occurs before changes in colour.

The excised embryo test relies heavily on the skill of the analyst, and embryos must be rejected if they become damaged. Refer to the International Seed Testing Association (1993) for detailed procedures.

Respiration measures

Oxygen use (respiration) is relatively easy to measure in the laboratory, and is a direct indicator of the physiological state or health of the seed. To determine respiration rates, seeds are incubated in a phosphate buffer solution (to maintain seeds in good physiological condition) in a temperature-controlled cuvette. Respiration is measured as a function of the rate of oxygen depletion of the solution. For further information on the significance and determination of seed respiration, refer to Leadem (1993).

7.3 Field Tests of Tree Seed Germination

Evaluation of germination success in the field requires introducing a fixed number of seeds to a microsite, then counting the number that germinate over some time interval. Some questions may lend themselves to modifications of laboratory methods for studying seed germination (Section 7.2), but because experimental conditions are so difficult to control, field trials are not suitable for investigating the physiological mechanisms affecting germination. The greatest advantage of field tests is that germination responses to treatments can be directly observed under field conditions, providing a better understanding of the ecological implications of the treatments being applied.

7.3.1 Experimental design and analysis

Field germination trials may evaluate treatments implemented at the stand level (e.g., canopy thinning), or at the level of individual germination plots or microsites (e.g., seedbed types). Care must be taken to recognize how treatments are nested and replicated. The layout of treatments and monitoring plots must be appropriate for statistical tests to answer the questions posed, and statistical tests must match the experimental design.

Plots should be distributed in unbiased and representative locations within uniform sites (see Section 1.6 for discussion of site selection). Variability can be high, and (despite preventative measures) some animal damage can be expected. A rough estimate of 10–30 plots per treatment combination can be used, although you should determine your own sample size needs based on the variability of the site (Section 1.4.2). When testing treatments, such as different site preparation methods, it is important to have plots in multiple independent replications of each treatment, not just in one stand or cutblock; this will help avoid a problem of pseudoreplication. Statistical analysis of experimental factors (e.g., seedbed type, site preparation method, silvicultural system) can be performed using analysis of variance. Continuous independent variables (e.g., duff thickness, canopy opening) are best assessed by regression analysis. Experimental factors and continuous variables can be combined in analysis of covariance.

The purpose of many field germination studies is to test for different degrees of canopy influence, and for this purpose, germination plots may be installed under different forest cover treatments. Canopy effects may be sampled over a range of random or fixed points (e.g., various distances from a forest edge) differing in their subcanopy light regime, thereby lending themselves to analysis by correlation or regression methods. Alternatively, canopy treatments may represent separate stand treatments (e.g., different silvicultural systems) which would be tested by ANOVA. Sample points should be situated randomly within the treatment blocks, and the canopy characteristics of each microsite should be documented using the appropriate cover- or light-measuring methodologies described in Section 2.3. Even if the primary interest is the response to treatments at the stand level, high spot-to-spot variation in canopy effects makes it desirable to collect enough microsite information to analyze plot-to-plot variation as well (e.g., by regression analysis or analysis of covariance).

7.3.2 Delimiting the site

One of the first requirements for evaluating field germination is a means of delimiting the microsite under study, and to contain the seeds being tested, so that sampling of sprouted seeds is limited to those introduced to the site.

One approach is to spread and evaluate seeds only within a fixed radius (e.g., 30 cm) from a tagged centre pin, or between two pins marking the diagonal corners of a square or rectangular quadrat that is placed on the plot only during establishment and monitoring. This results in less impact to the microenvironment, but with no protection, the seeds may be washed away or removed by birds and rodents.

Another option is to set out a rigid circular or rectangular plot perimeter (e.g., using plastic lawn edging or wood) (Figure 7.7). This method constrains the movement of seeds, but may modify the microclimate. A tall, solid frame may alter subtle microsite effects, so frame height should be kept minimal. Smaller frames may be more suitable for testing smaller microsites, such as those prepared by mounding.

Seed samples should be located within the plots, but away from the edge. Several tree species or seedlots can be tested in the same plot, although interior fences of interlocking metal window screening are recommended to keep different subsamples of seeds

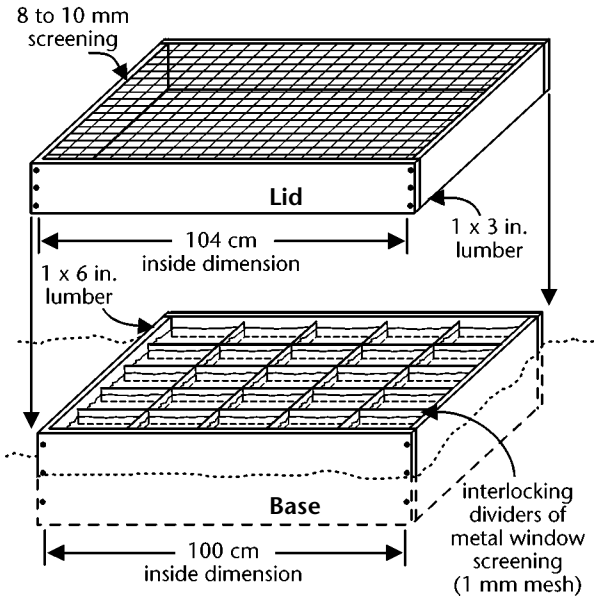


FIGURE 7.7 *A recommended frame design for delimiting field germination plots. This design is effective in constraining the movement of subsamples within the plot, and excluding seed predators.*

from becoming mixed. This is especially important if the plot is not level, is subject to flooding, or if animals move the seeds.

7.3.3 Excluding other seeds

You should check to ensure that the experimental site has not been contaminated by natural seed rain. Seed rain from most conifers can be considered negligible if the plots are located more than three tree-heights from mature trees. But when testing germination of hardwoods that disperse over long distances (e.g., aspen, cottonwood, birch, alder), seed rain contamination cannot be ignored, even in the middle of a large clearcut. During the season when they are dispersing, seeds can be excluded with nylon window screening, cheesecloth, or remay (which allows rainfall penetration); coverings can be removed after a fixed number of test seeds have been introduced. Such protective devices cannot validly be left in place during the actual germination experiment, because they can potentially modify the micro-environment to a degree that the plot is no longer representative of the microsite being tested. Alternatively, equivalent plots (identical area and seedbed makeup) can be left unsown, and all germinants counted, and the mean density used to adjust the germinant counts in the plots with the introduced seeds.

The degree of error due to physical loss (as opposed to biological loss) of seeds can be quantified by placing randomly on the site several replicates of glass beads the same size as the seeds. Refer to Johnson and Fryer (1996) for additional details.

7.3.4 Preparing the seeds

The seeds used for germination trials should be locally collected, or obtained from seed sources matched as closely as possible to ecologically similar sites and within the same relatively narrow geographic and altitudinal range. It is inappropriate to conduct studies (and extrapolate results) when seeds are not genetically adapted to the specific site in which they are sown.

Seeds can be placed in experimental plots directly after collection. Note, however, that in commercial seed collections, the seeds are usually dewinged during processing. Also, if the seeds have been prepared for nursery sowing, they may have been stratified before shipping. These practices may be acceptable if you are testing spot-seeding methods and the same practices will be used in operational situations. However, if you are interested in evaluating natural seed rain, you may not want the seeds subjected to any treatments before sowing.

Seeds scattered on the surface of the seedbed sometimes cannot germinate because they are perched on obstacles or otherwise have poor contact with the substrate material (though they may eventually be washed in by rain). If the direct effects of the seedbed and the microsite are of greater interest than documenting natural losses to the seed population, push the seeds into the substrate or sprinkle some material on top of the seeds. This approach may result in higher germination levels, but it is rather artificial.

If unstratified seeds are used, it is better to sow them during the season in which they would naturally be dispersed (e.g., fall for most conifers, spring for *Populus* spp., late spring or early summer for aspen and willows) so they can meet their stratification and/or germination requirements over the natural progression of seasons. Unstratified conifer seeds might also be sown in spring, but stratification may be inadequate under such conditions (e.g., Dominy and Wood 1986).

If stratified seeds are used, they must be sown as early as possible in the spring. If the seeds are not present on the site before the time they would ordinarily emerge, they may not follow the same germination pattern as naturally dispersed seeds (J. Zasada, pers. comm., 1996). High germination rates generally can be expected when stratified seeds are sown in spring.

7.3.5 Excluding predators

Losses to seed predators are often the most serious obstacle to obtaining good field information on seed germination (Section 5.2), especially for relatively large-seeded species, such as Douglas-fir, ponderosa pine, grand fir, maple, and oak. The use of predator odours is sometimes an effective deterrent, but providing highly palatable alternative foods (e.g., sunflower seeds) can reduce—though not eliminate—losses of conifer seeds to small mammals (Sullivan 1979a).

Offering alternative foods may be suitable for direct seeding operations, but may not be an option for experimental studies where experimental seed losses are unacceptable. Full physical exclusion is generally the best approach. Devices range from small plastic berry baskets to cone-shaped hardware-cloth tents (Frenzen and Franklin 1985; Mitchell et al. 1990; Adams and Henderson 1994). See Section 5.4 for a full description of exclusion devices. The mesh must be small enough to exclude deer mice without detectably modifying the microclimate; 8–10 mm mesh is generally adequate. The lower flange must also be anchored to the soil and preferably buried to prevent rodents burrowing under to get the seeds. The best exclosures are large wooden frames (30–100 cm on a side) similar to the seed traps described in Section 4.3. The lower frame can be buried in the soil, with a tight-fitting screened lid resting on top (Figure 4.4a). The lid can be removed for monitoring and, to minimize internal shading, the entire unit can be set flush with the ground surface.

Instead of excluding seed predators from germination plots, many researchers consider them to be part of the local environment and do not try to exclude them. Zasada et al. (1983) introduced seeds of several conifer and hardwood species to unprotected burned seedbeds and then monitored net germination (emergence) and early survival. Similarly, Burton and Bazzaz (1991) introduced unprotected hardwood seeds into different vegetation types, and noted different degrees of seed predation among vegetation types. In some cases, a more realistic estimate of a site's regeneration potential might be obtained by calculating seed-to-seedling ratios from data gathered from unprotected sites (e.g., Noble and Ronco 1978; Alexander 1986; Cain 1991; McDonald and Abbott 1994).

7.3.6 Monitoring germinants

The survival of newly germinated seedlings is often examined in association with germination studies. Monitoring of germination is usually required to capture the natural time span in which germination occurs, which for many habitats in British Columbia may be spread over 6–15 weeks. Simply counting germinants at the end of the growing season is not recommended (unless you are primarily interested in net recruitment) because many seedlings die and may be largely decomposed before the end of the season (Gashwiler 1971). Monitoring at intervals of 2–4 weeks is recommended, with all germinants removed or tagged at each interval. For species such as willows and aspen, whose seeds germinate within 1–2 days and all germination occurs in a 2-week period, monitoring at 1-week intervals or more often will be required.

Differential tagging of new germinants at each monitoring interval (i.e., each cohort) is desirable, because cohorts may differ in their susceptibility to drought and pests. Researchers often use coloured toothpicks or plastic cocktail swords to mark individual seedlings (e.g., Brown et al. 1988), but these become impractical if germinant densities are high, or if frost-heaving is a problem. Wire hoops are preferred because hoops are more difficult to displace than markers stuck into the ground. Plastic-insulated coloured wire (telephone wire or garden twist-tie wire) can be cut into 2–3 cm lengths and looped around the base of each seedling. Other options include tags used for banding birds, and sections of slit plastic straws, which can be numbered with waterproof ink.

7.4 Experimental Design for Germination Studies

Most environmental studies involving tree seeds are designed to compare how different treatments affect

seed germination. Germination test conditions and procedures are often governed by seed testing rules (Association of Official Seed Analysts 1993; International Seed Testing Association 1993), at least for many laboratory experiments. Regardless of whether or not tests are conducted under standard conditions, once the test environment has been determined, the next task in designing an experiment is to determine which factors (and levels of those factors) will be selected for study.

7.4.1 Experimental factors

A factor is a variable that may affect the response of an experiment, and has, in general, more than one level. A particular factor is chosen because the experimenter wants to test or compare how the different levels of that factor affect the measured response. A factor can be classified as either fixed or random, depending on how the levels are chosen. A fixed factor has levels that are determined by the experimenter. If the experiment is repeated, the same factor levels would be used again because the experimenter is interested in the results for those specific levels, and the results would be applied only to those levels. A random factor has levels that are chosen randomly from the population of all possible levels. If the experiment is repeated, a new random set of levels would be chosen. The experimenter is interested in generalizing the results of the experiment to a range of possible levels and not just to the levels selected (Sit 1995). For example, the factor "seed source" is considered *random* if the sources are chosen from the full range of sources available in the province, and if the purpose of the experiment is to extend the results to the species as a whole. However, to examine the effects of latitude on germination, and seed sources are chosen from particular latitudes to test this effect, then the seed source factor is considered to be *fixed*.

The factor and levels of a factor chosen for comparison depend on the underlying objectives of the study. In general, a particular species is selected for study because the species may exhibit difficulties with germination or dormancy (e.g., *Abies* requires exceptionally long stratification to germinate well) and information on the specific germination behaviour is required. Species could be selected by their presence in a particular biogeoclimatic zone (e.g., coastal versus interior), or their position in seral succession (e.g., pioneer versus climax). Species could also be selected if one has an interest in the responses of different species to a particular variable, such as temperature.

Choosing different seed sources generally implies that one is interested in the effects of genetic variation. For example, seed source may be included as an experimental factor if one wants to compare the differences between northern and southern populations of the same species, between coastal and interior seed sources, or between low-elevation and high-elevation seed sources.

There are two main groups of seed treatments: (1) treatments that are applied before the germination test, and (2) treatments that are applied during the germination period. Some examples are as follows:

1. Treatments applied before the germination test:
   a) dormancy release treatments (e.g., stratification, scarification);
   b) upgrading treatments (e.g., specific gravity separation, polyethylene glycol);
   c) cold storage conditions (e.g., number of years stored; storage at +2°C versus 18°C); and
   d) seed moisture content (e.g., 25% mc versus 45% mc).
2. Treatments applied during the germination period:
   a) environmental conditions (e.g., temperature, moisture stress); and
   b) daylength or thermoperiod (e.g., different day/night hours, different day/night temperatures).

7.4.2 Experimental designs

Single factor

An experiment involving a single factor has a one-way design. Depending on how the factor levels are assigned to the experimental units, the design could be completely randomized, or a randomized block. In a completely randomized design, there are many homogeneous experimental units; each experimental unit is randomly assigned to one of the factor levels. Each factor level often has an equal number of experimental units, or replications, although such a balanced design is not necessarily required. If the experimental units are not homogeneous, they could be arranged into groups or blocks, according to some common characteristic (e.g., location, aspect) so that

the variation within a block is smaller than the variation between blocks. Within each block of experimental units, factor levels are assigned to the experimental units at random. In essence, a randomized block design has several blocks of experimental units, with a completely randomized design within each block. (See Figure 7.8 for an example using two levels of one factor.)

Multiple factors

Carefully choose the blocking criteria. Since the experimental units within individual blocks are different, their responses to the factor levels are expected to be different. However, the relative responses to treatment effects should be consistent from block to block. For example, to compare the effect of two different daylengths on seed germination rate of lodgepole pine, a randomized block design could be used with coastal and interior seed sources being selected as the blocking criteria. This is legitimate if seed source does not alter how daylength affects germination rate. That is, the longer daylength should be more (or less) effective than the shorter daylength for both coastal and interior seed sources. If, however, seed source alters the effects of daylength (for example, longer daylength increases rates of the coastal seed source, but decreases rates of the interior seed source), then seed source is not a suitable blocking criterion. If how seed source will influence the effects of daylength on germination is not known, or if the goal is to find out how the two factors—seed source and daylength—interact with each other, then a completely randomized design involving both factors should be used.

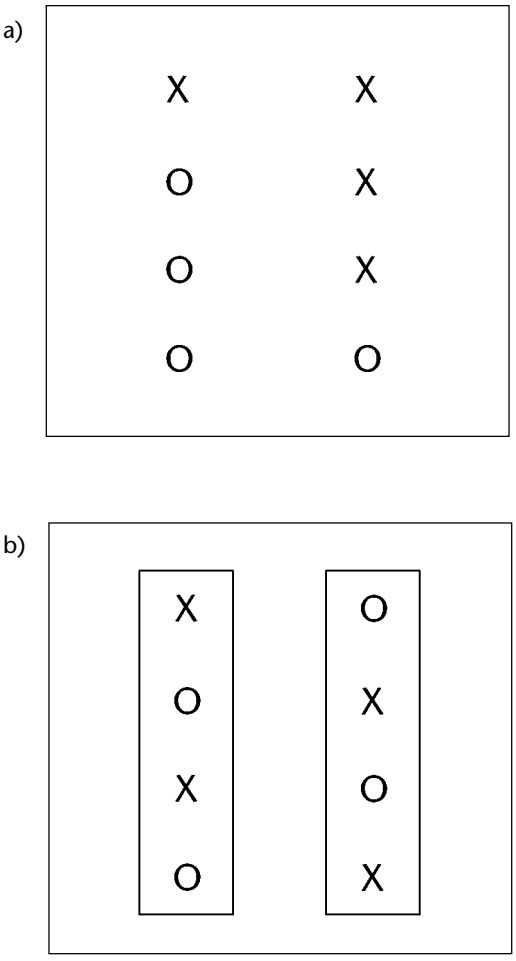


FIGURE 7.8 *Layouts for one factor with two levels (X = 8-hour daylength, O = 16-hour daylength): (a) completely randomized design, and (b) randomized block design.*

In a factorial design experiment, more than one factor is involved, and all levels of one factor are combined with all levels of the other factors. In the daylength–seed source example, a factorial experiment could be designed to assess the effects of daylength and seed source on germination rate. Each experimental unit would be randomly assigned one level of the daylength treatment and one level of the seed source treatment. The assignment of one factor level to an experimental unit should not affect the assignment of any level of the other factor. The primary advantage to using a factorial design is the ability to examine both daylength and seed source effects on seed germination, plus the combined effects of daylength and seed source. A factorial experiment may be carried out in a completely randomized or randomized block design.

Assigning the factor levels to the experimental units completely at random is easy to accomplish. However, it is sometimes advantageous to do the random assignment at several levels. For example, consider an experiment that examines the effects of cold storage conditions (+2°C versus -18°C) and daylength (8-hour day versus 16-hour day) on seed germination (Figure 7.9). Four hundred seeds are available for the experiment. In a completely randomized factorial experiment, *each* seed would be randomly assigned one of the four treatment combi-

nations. In this case, an individual seed is the experimental unit for the factors cold storage condition and daylength. However, it may not be efficient to assign treatment combinations to each seed. Since the cold storage treatment must occur before the daylength treatment, the experiment could be designed differently. The seeds could be grouped into 10 trays of 40 seeds each, and each tray could be assigned to one of the two cold storage treatments (five trays at +2°C and five trays at -18°C). Each tray of 40 seeds represents one experimental unit of the cold storage treatment (Figure 7.9 a).

After cold storage treatment, each tray of seeds could be subdivided into two groups of 20 seeds. Within a tray, one group of seeds would be exposed to 8 hours of daylight, the other group would be exposed to 16 hours of daylight. The assignment of daylength to the two groups of seeds is completely random. The experimental unit for the daylength treatment now consists of a group of 20 seeds. This is a split-plot design with cold storage as the main plot treatment and daylength as the split-plot treatment (Figure 7.9b).

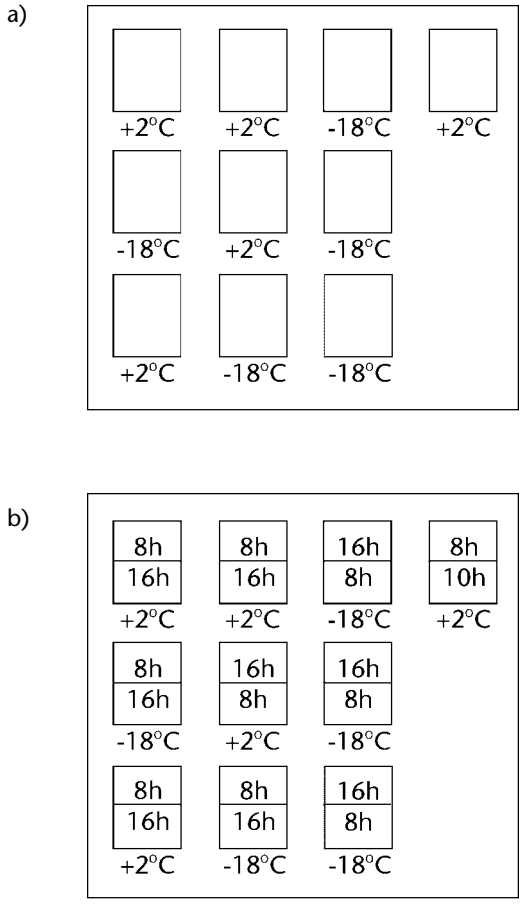


FIGURE 7.9 *The two stages of a split-plot design (see explanation in text).*

A split-plot design has the characteristic that the two treatment factors have different experimental units, and the experimental unit for the split-plot factor is contained within the experimental unit of the main-plot factor. The randomization of the split-plot factor is restricted in that both levels of daylength treatment must occur within each tray of seed. In addition to ease of execution, the split-plot design also yields more precise information on the split-plot factor, but at the expense of losing information on the main-plot factor. For further discussion of these and other types of experimental designs, consult Anderson and McLean (1974); Steel and Torrie (1980); Sokal and Rohlf (1981); Mead (1988); Milliken and Johnson (1992); and Sit (1995).

7.4.3 Replication and randomization

An experimental unit is the smallest collection of the experimental material to which one level of a treatment factor may be applied. Germination tests, for example, are usually conducted on groups of 100 seeds; for such tests, a group of 100 seeds represents one experimental unit. A replication is an independent application of a factor level. A factor level is considered to be replicated if it is applied to two or more experimental units. The number of replications of a level is the same as the number of experimental units to which a factor is assigned (Sit 1995). For example, if each factor level of a germination experiment was assigned to four experimental units, then the experiment would have four replications. Replication quantifies the size of the experimental error, so that treatment factors can be properly compared. An unreplicated study has weak comparison power and can only be viewed as a one-time occurrence for which the results cannot be generalized. Differences observed in an unreplicated study could be due to the treatment, or to random variation; without replication it is not possible to determine the cause of the observed variation.

The number of replications required to detect differences between treatments will depend upon the

variability of the experimental material. Seed testing rules (Association of Official Seed Analysts 1993; International Seed Testing Association 1993) prescribe four replications of 100 seeds for germination tests, because conditions for laboratory tests are relatively uniform. More replicates are required for nursery and field tests. Nursery-test results tend to be more variable than laboratory tests, because of changing conditions within greenhouses (e.g., shading, drying of blocks near edges of watering booms). In the field, tests are subjected to all the vagaries of field environments: seeds may be washed or blown away, eaten by predators, or simply lost (hard to tell a small dark seed from a small dark piece of soil).

When only a limited number of seeds are available, such as in experiments using seeds derived from controlled genetic crosses, the number of seeds per replication may have to be reduced to achieve sufficient replication to determine differences between treatments. The minimum number of seeds per replication depends upon the variability of the test material. For tests conducted in closed dishes in controlled environment chambers, for example, 25 seeds per replication might be used and still assure detection of statistical differences. It is advisable, however, to increase the number of replications (perhaps from four to six) whenever the number of seeds per replication must be reduced.

For germination tests conducted in controlled environment chambers, the issue arises as to whether controlled environment treatments are truly replicated when only one chamber is used for each experimental condition. More than one chamber per condition must be used to achieve true replication. The cost of controlled environment equipment generally prohibits the use of more than one chamber, but in reality, use of only one chamber per condition constitutes pseudoreplication. In most tree seed studies, however, the variance due to the effects of treatments (i.e., stratification, seed source) is usually far greater than the variance due to within-chamber variation. Nonetheless, pseudoreplication should be acknowledged when presenting and interpreting results.

Randomization is required for sound experimentation as it helps to reduce systematic bias within the experiment. Randomization should occur at several levels: random selection of experimental units for treatment assignment, random assignment of treatment levels to the experimental units, and, where necessary, random selection of sampling units within an experimental unit for measurements.

7.5 Data Analysis in Germination Studies

The method of analysis depends on the design of the experiment and the type of data collected. For continuous data such as percent germination, analysis of variance (ANOVA) and regression are suitable analysis methods. For discrete data, such as germination status (germinated, not germinated), categorical data analysis methods such as a chi-square test are more appropriate.

7.5.1 ANOVA

Understanding the proper application of error terms is essential when using ANOVA for data analysis. A common mistake is to use the experimental error term to test all effects. The experimental error is only suitable for testing when the design is simple. In a randomized block design, for example, main effects are often tested by the block-by-treatment interaction terms. The following two examples show the error term structure in ANOVA tables for a one-factor completely randomized design and a two-factor randomized block factorial design.

One-factor completely randomized design

To comparetwo levels of soil storage conditions (1 year versus 5 years), 10 bags of 100 seeds are buried underground. At the end of year 1, five bags of seeds are randomly pulled out of the ground for germination. The remaining 5 bags of seeds are assessed at the end of year five. The percentage of seeds germinated is recorded for each bag. A bag of seeds is the experimental unit for soil storage treatment.

ANOVA TABLE

Source of variation	Degrees of freedom	Error term
Treatment, T	t-1 = 2-1 = 1	$B(T)$
Bag, $B(T)$	$(b$-1$)t$ = (5-1)(2) = 8	—
Total	$(b)(t)$-1 = (5)(2) - 1 = 9	

The term $B(T)$, usually denoted as the experimental error, is the correct error term for testing soil

storage treatment effects. Note that the total degrees of freedom in an ANOVA table should be one less than the total number of measurements in an experiment.

Two-factor randomized block factorial design
To compare the effects of two stratification treatments (e.g., 3 weeks, 6 weeks) and three germination temperatures (e.g., 10°C, 20°C, 30°C) on white spruce seed germination, seeds from four different seedlots, randomly selected from all available seed sources, are used in a study. Six batches of 100 seeds are available from each seedlot. For each seedlot, one of the six stratification-temperature treatment combinations will be randomly assigned to an individual batch of seeds. It is expected that the stratification–temperature effects on white spruce seed germination are consistent across seedlots, hence seedlot is acting as a block in this design. A batch of seeds represents the experimental unit for the stratification and temperature treatments. Percent seed germination is recorded for each batch of seeds.

ANOVA TABLE

Source of variation	Degrees of freedom	Error term
Seedlot, *L*	*l*-1 = 4-1 = 3	—
Stratification, *S*	*s*-1 = 2-1 = 1	*L*S*
Temperature, *T*	*t*-1 = 3-1 = 2	*L*T*
*S*T*	(*s*-1)(*t*-1) = 2	*L*S*T*
*L*S*	(*l*-1)(*s*-1) = 3	—
*L*T*	(*l*-1)(*t*-1) = 6	—
*L*S*T*	(*l*-1)(*s*-1)(*t*-1) = 6	—
Total	(*l*)(*s*)(*t*)-1 = 23	

The term *L*S*T* is often called the experimental error. However, it is only the correct error term for testing stratification-temperature interaction (*S*T*). Stratification (*S*) and temperature (*T*) effects are tested by the corresponding seedlot interaction terms (*L*S* and *L*T*) because *S* and *T* are replicated by randomly chosen seedlots. The terms *L*, *L*S*, *L*T*, and *L*S*T* are not testable because these terms include the seedlot factor which is not replicated (there is only one of each seedlot).

ANOVA assumes that residuals must be independent, normally distributed, and have equal variance. These assumptions must be checked to ensure the analysis is appropriate. See Section 3.7.3 for more discussion of ANOVA assumptions. For ANOVA tables for other designs, see Chapter 6 of Sit (1995).

7.5.2 Categorical data analysis

If the data collected in an experiment are categorical (e.g., the degree of mould infestation, see Table 7.2), then ANOVA is not suitable for the analysis. Categorical analysis methods such as the contingency table chi-square test allow you to check whether the proportions of seeds in the classes are similar for the treatments. Refer to Sections 5.5 and 8.3.4 for examples of categorical data analysis.

7.5.3 Regression

Regression is a common method for modelling the relationship between several variables. In seed germination studies, you may want to model the rate of germination over time. Since the shape of the curve relating percent germination and time is not a straight line, nonlinear regression should be used for modelling the data. The exponential Gompertz function is particularly suitable for germination data (Tipton 1984). Cumulative germination percentage can be fitted to the Gompertz curve of the form:

$$Y = A \exp\left[-\exp(B - Ct)\right],$$

where:

Y = cumulative germination percentage at time t;
exp = exponential function;
A = parameter corresponding to the final germination percentage;
B = parameter reflecting the start of germination;
C = parameter indicating intrinsic rate of growth (i.e., germination speed); and
t = time (e.g., number of days or number of weeks).

In regression, germination data points at various times t are used to estimate parameters A, B, and C. The usefulness of the fitted regression model depends on the suitability of the chosen function. A general

rule for nonlinear regression is to use the simplest model that best fits the data. For ease of interpretation, the form of the model should also be compatible with the underlying biological mechanism driving the system. For example, both an exponential function and a polynomial function have an increasing trend. However, the exponential function is more suitable for modelling growth data, because growth is well understood (biologically) to be exponential in nature. Thus, parameters expressed in an exponential function have more meaningful interpretations than parameters expressed in a polynomial function. For a full discussion of commonly used models for nonlinear regression and practical hints for fitting nonlinear models, see Ratkowsky (1990) and Sit and Poullin-Costello (1994).

Nonlinear regression can also be used as a tool for comparing trends (e.g., comparing the germination trends of seeds receiving different treatments or of different species). The analysis would begin with estimating the model parameters (A, B, and C) for each replication of the treatment factor. The parameter sets thus estimated would then be used as data in a multivariate analysis of variance (MANOVA). If the MANOVA is significant, then univariate ANOVA would be done on each parameter separately to determine which parameters are significant. This curve-fitting approach to comparisons has several advantages. It appropriately takes into account the time-to-time correlation in the data. By reducing the data to a few parameters, it simplifies the MANOVA. Also, with the appropriate choice of a model, features of the germination curve that are of most interest to the experimenter (e.g., time when germination is first observed, maximum germination speed, and final germination percentage) can be directly compared. Examples of this type of data analysis can be found in Leadem (1986); Sit (1992b); and Stoehr et al. (1994).

SECTION 8 SILVICULTURAL PRACTICES AND TREE SEED BIOLOGY

Some seeds fell by the wayside, …some fell upon stony places, …and some fell among thorns; …but others fell into good ground …
(The Bible, Matthew 13:3–8)

8.1 Background

Tree seed studies are often conducted or repeated under field conditions to better understand the significance of seed ecology in forest regeneration. The incentive for much forestry research on seeds is directly related to testing alternative silvicultural treatments that facilitate natural regeneration. This section will briefly review forest practices that affect regeneration by seeds, and offer some suggestions for studying seed dynamics under field conditions.

8.1.1 Principles of forest stand manipulation

In silviculture, the environmental factors discussed in Section 2 are manipulated to enhance the regeneration, establishment, and growth of desired tree crops. Most silvicultural practices are designed to alter either the canopy (the above-ground growing space), or the seedbed (the substrate for seedling establishment and the below-ground growing space).

Promoting forest regeneration from seeds requires a favourable combination of seed supply, seedbed, and environmental conditions (Figure 8.1), so many silvicultural practices are designed specifically to enhance these factors. Silvicultural practices may range from specialized procedures to induce tree seed production to the multipurpose practices of canopy opening and forest floor disturbance. Should these activities be unable to regenerate the desired tree species at target stocking levels, then specific site preparation operations are undertaken to create or improve microsites so they are more suitable for tree seedling establishment.

Regeneration silviculture is based on two important principles of forest ecology:

- Some level of disturbance (natural or artificial) is usually required to free plant growth resources and provide growing space for new trees (Bazzaz 1983; Canham and Marks 1985).
- Seed germination and seedling emergence are crucial steps in the life cycle of many plant species, and only a narrow range of conditions is suitable for seedling establishment (Grubb 1977; Harper 1977; Oswald and Neuenschwander 1993).

Much of the experimentation and monitoring associated with silvicultural research is therefore concerned with assessing the degree of disturbance achieved, or with evaluating the success of young trees in a variety of natural or modified microsites.

8.1.2 Standard silvicultural practices

Forest management activities are implemented over wide areas and long time periods. Timber harvesting, road development, resource zoning, and fire protection policies all affect large areas of land at the forest or landscape scale. However, to researchers studying seed ecology, activities performed at the stand level are more relevant. Typical stand-management activities may include resource inventories, protection from wildfire and pests, timber harvest, and stand renewal. Some activities, such as inventories, have no effect on a stand, while other activities, such as logging or prescribed burning, can be extremely disruptive. The fundamental principles of sustainable resource development and environmental protection assume

that, whatever the type of stand manipulation, all activities should be conducted in a manner that facilitates stand maintenance, rejuvenation, or restoration.

A silvicultural system is a set of stand management treatments for forest tending, harvesting, and replacement. Except for a brief overview, it is beyond the scope of this manual to describe the range of practices that comprise different silvicultural systems (see, for example, Matthews 1989). Each silvicultural system is composed of many individual components, and each component requires testing and modification to be applied effectively in a given forest type. Individual stand management treatments may be prescribed to alleviate some limiting factor, but in practice, numerous treatments usually are enacted as a package.

The amount of mature canopy retained during regeneration of a stand (or the nature of the first stand entry into the stand for timber harvest) is typically used to describe the entire silvicultural system. In each system, the availability of tree seeds is largely determined by the density and distribution of the residual mature trees left in the stand. Five silvicultural systems used at the Lucille Mountain Project are illustrated in Figure 8.2.

- A clearcut system creates stand openings that are dominated by exposed, full sunlight conditions.
- A seed tree system is similar to a clearcut system in that it creates a microclimate characteristic of open conditions, but a few scattered trees are retained to provide seeds for natural regeneration.
- In shelterwood systems the density of residual trees is greater, so that the retained overstorey provides protection (from sun-scald, frost, etc.) to the regenerating tree crop.

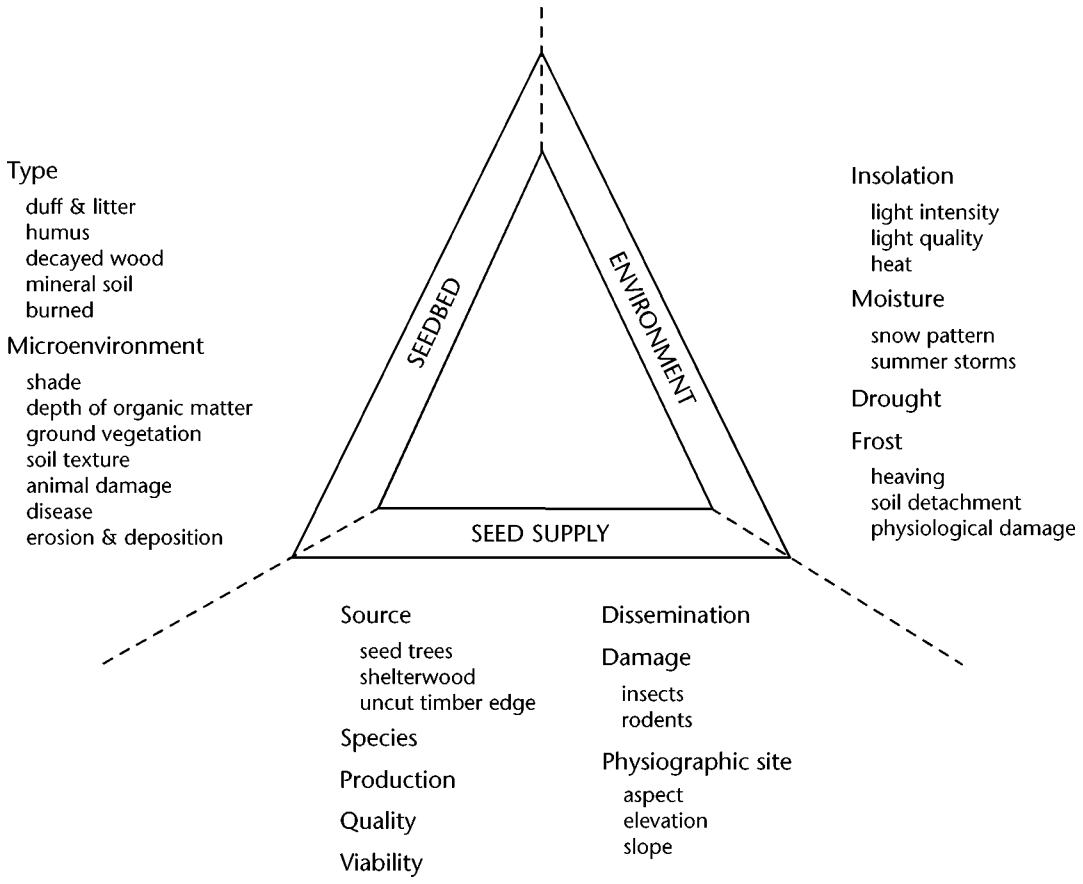


FIGURE 8.1 *Effective natural regeneration depends on an adequate seed supply, a suitable seedbed, and an appropriate environment. All sides of this "natural regeneration triangle" must be adequate to achieve successful natural regeneration (from Roe et al. 1970).*

- Clearcut, seed tree, and shelterwood systems regenerate even-aged stands. In selection systems, single trees or small groups of trees are harvested to regenerate the forest in smaller canopy gaps, maintaining continuous forest cover and an uneven-aged structure to the tree population.

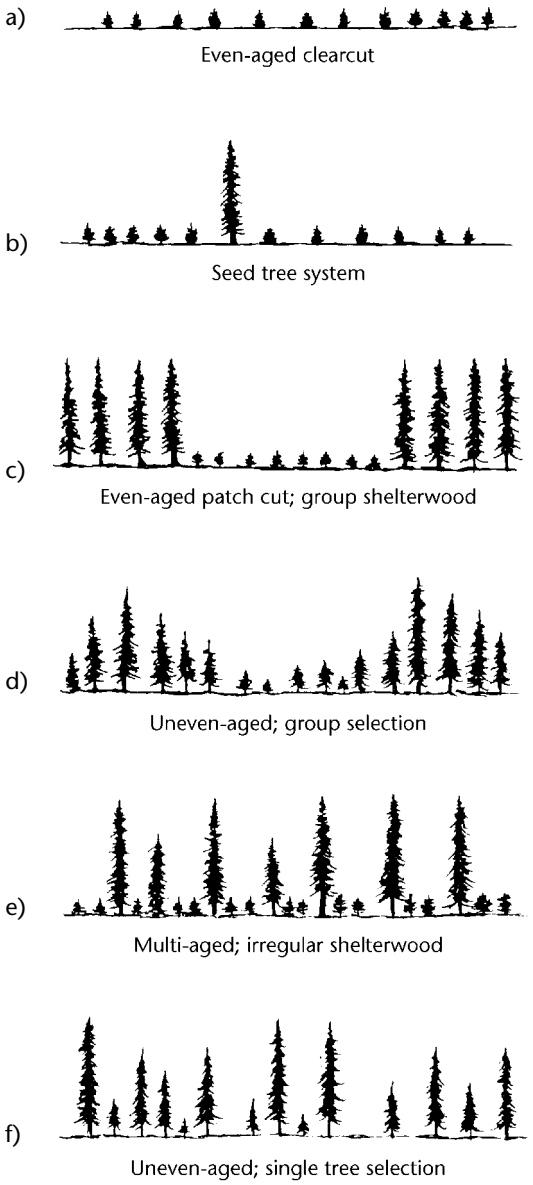


FIGURE 8.2 *Illustration of stand structure resulting from different silvicultural systems used in the Lucille Mountain Project in the Engelmann Spruce–Subalpine Fir (ESSF) biogeoclimatic zone, Prince George Forest Region, British Columbia (adapted from Jull et al. 1996).*

Silvicultural systems were originally designed to facilitate natural regeneration, usually by seeds. However, if natural regeneration proves unsuccessful or too unpredictable, dependence on natural seed availability can be bypassed through artificial regeneration, which introduces seeds or seedlings for stand renewal. Direct seeding can be undertaken in a broadcast manner (often from aircraft), but it is more effective if efforts are focused in spots or strips that have been prepared to favour germination and establishment (Mitchell et al. 1990). By planting seedlings, germination and early growth occur in the generally more favourable environment of forest seedling nurseries. Coppice systems count on the vegetative sprouting of stumps or roots (e.g., the profuse suckering of trembling aspen after harvest), which also bypasses the greater risks of reproduction and establishment of hardwoods from seeds.

Successful seedling establishment is a good indicator of the degree of canopy opening and forest floor disturbance that can be tolerated without degrading the ecosystem. For experimental purposes, some silvicultural practices may be performed and monitored on a relatively small scale—on research plots rather than on the entire stand. Treatments might include mounds made by hand or burning slash and forest floor with a propane torch. However, care should be taken when extrapolating the effects of microscale treatments to the probable effects of macroscale disturbance. The disturbance created by large equipment on soil properties, for example, is not equivalent to the effects created by hand equipment. Many treatments only produce reliable results if they are implemented with large machines over several hectares.

8.2 Effects of Canopy Manipulation

A complex of irradiance, temperature, humidity, wind, and other microclimate factors are associated with the shading and sheltering influence of forest canopies. These factors change in proportion to the degree of canopy opening in silvicultural systems. Indeed, silvicultural systems are classified in large part according to the degree they open the canopy in the first, or regeneration, cutting (Matthews 1989; Klinka et al. 1994).

The removal of part or all of the tree canopy releases light, water, and nutritional resources for seeds and seedlings, but it also often results in development of ground and shrub canopies that impose more restrictions than the original overstorey. Some interactions with other canopy-related factors may be difficult to anticipate, for example, the effects of one canopy layer on another layer, or the effects of different vegetation layers on the same microsite.

8.2.1 Light

The most immediate and dramatic effect of tree harvesting is increased light levels in the understorey (Figure 8.3). Ecosystems vary in the degree to which increased light levels stimulate the growth of understorey (shrub and herb) vegetation, and such growth may negate in a few years the effects of canopy opening on the forest floor. The increased amount of light caused by total canopy removal may stimulate the rate of germination of species such as pines (Li et al. 1994). For other species, partial canopy removal may be more favourable to germination and early survival because retaining partial canopy cover helps moderate the moisture and temperature of the seedbed. Protected microsites that maintain higher-than-ambient levels of humidity may not support optimal seedling growth, but still are important for seed germination and emergence (Frenzen and Franklin 1985; Battaglia and Reid 1993). Partial canopy cover provides protection from sun scalding, photoinhibition, desiccation, and radiation frosts that might critically affect the survival of young, unlignified seedlings.

8.2.2 Temperature

Canopy removal may have a greater influence on the temperature than on the light properties of microsites (Figure 8.4). Total forest canopy removal results in higher (and potentially lethal) soil temperatures and more extreme temperature fluctuations (Hungerford and Babbitt 1987; Stathers and Spittlehouse 1990), both of which can negatively affect seed germination and seedling survival. On the other hand, complete canopy removal and some forest floor disturbance are often beneficial at high elevations, high latitudes, or on northern exposures where soil temperatures may be too cold for germination (Bonan 1992; Balisky and Burton 1995).

8.2.3 Moisture

The most usual immediate effect of canopy removal is a rise in the water table; the loss of tree cover reduces transpiration and increases moisture in the root zone. However, the removal of a tree canopy exposes the soil surface to direct sun, to high surface temperatures, and to increased wind speed, and this can result in rapid drying of the surface layers that constitute the seedbed. These conditions often arise

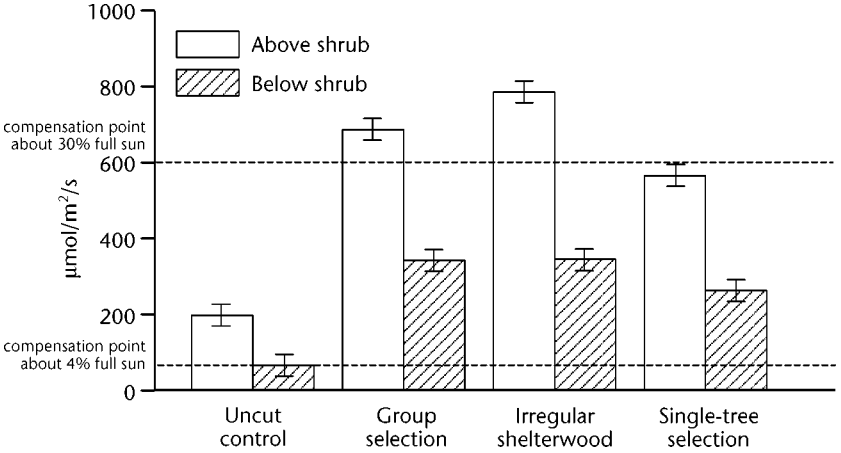


FIGURE 8.3 *Measured levels of photosynthetic active radiation (PAR) available to seedlings above and below the shrub layer in various partial cut systems at the Lucille Mountain Project, Prince George Forest Region, British Columbia (Jull et al. 1996). Results are shown for treatments illustrated in Figure 8.2d, e, and f.*

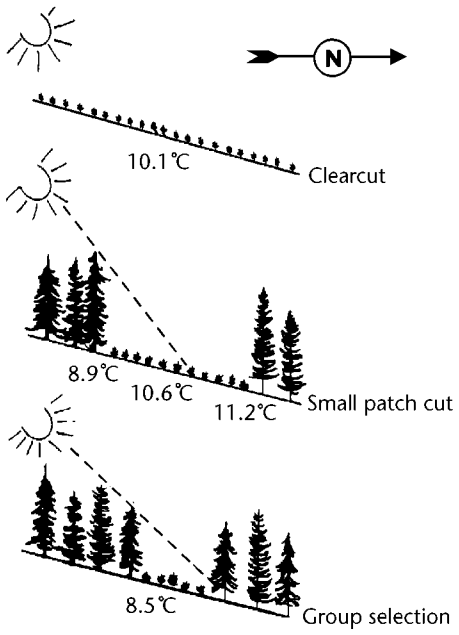


FIGURE 8.4 *Growing-season soil temperatures (at 10 cm depth, 4-year means) on a north-facing slope in clearcut, small patch cut, and group selection treatments (Jull et al. 1996). Results are shown for treatments illustrated in Figure 8.2 a, c, and d.*

after canopy removal in clearcuts, and historically, have been the major reason to develop silvicultural systems that provide better protection to seedbeds and to newly established seedlings.

8.2.4 Suggested questions and approaches

Monitoring of tree seed germination and early seedling population dynamics is often part of evaluating the effectiveness of silvicultural systems in promoting forest regeneration. Much of the incentive for choosing one silvicultural system over another is the expectation that one method is more effective in producing seeds or creating better conditions for germination and early seedling survival. In some stands, trees are retained to provide seeds or to protect young seedlings (such as seed tree, shelterwood, strip cut, and some patch-retention systems). Questions that might be asked in regeneration silviculture field trials include the following:

- Is cone production per tree greater in partially girdled trees than in untreated trees?
- Is seed rain density significantly greater under a shelterwood system than a seed tree system?
- Is tree seed germination more successful with or without some canopy cover? Does this vary by species and latitude?
- What is the seed-to-seedling ratio under alternative stand treatments?
- Can a given stand or stand treatment meet a desired level of stocking through natural regeneration?
- What level of canopy retention significantly reduces (or increases) early seedling survival?
- Does one tree species germinate and/or establish better than another under a particular canopy treatment (e.g., a shelterwood cut removing 50% basal area)?
- Do canopy effects on germination depend on seedbed conditions? For example, is shade better for germination on all substrates, or just for seeds germinating in the duff layer?
- What is the relation between canopy reduction, understorey development, and the light and resource levels of the seedbed?

The objective of many studies is to correlate indicators of successful natural regeneration with various canopy openings. For example, tree seedling establishment has been correlated with canopy density, subcanopy irradiance levels (e.g., Burton and Mueller-Dombois 1984; Stewart 1988; Canham et al. 1990; Denslow and Hartshorn 1994), specific environmental variables (Geier-Hayes 1987; Oswald and Neuenschwander 1993; Denslow and Hartshorn 1994), or site variables. Seedling establishment is usually enhanced in forest gaps or disturbances (Platt and Strong 1989; Coates and Burton [1998]), but under extreme environmental conditions better establishment may be found in protected, shaded microsites (Frenzen and Franklin 1985). Evaluating the success or failure of silvicultural treatments may depend on when the assessment is made (i.e., after one growing season or after 5 years). Successful establishment of seedlings may depend more on the ability of germinants to survive on a particular site than on the number of seeds that initially germinate (Burton 1997). Optimal conditions for seed germination are not necessarily optimal for plant survival and growth of the same species (Parrish and Bazzaz 1985; Oswald and Neuenschwander 1993; Schupp 1995).

Once relationships are noted, more detailed experiments may be undertaken to verify initial study results. This may be accomplished, for example, by studying the effects of canopy shading on tree seed germination in glasshouses or slathouses where shade levels can be controlled (e.g., Minore 1972; Alexander 1986). In this regard, note that "neutral shade" treatments created in slathouses are sometimes considered unrealistic because they do not mimic the spectral shift normally found under plant canopies. In neutral shade treatments, light passes through a filter with no change in the relative proportions of various wavelengths. Under plant canopies, the light spectrum has proportionately less light in the red and blue wavelengths and more in the green wavelengths (see Section 2.3.3. and Figure 2.4). Nevertheless, neutral shade is a useful experimental tool because it allows researchers to vary the quantity of light while maintaining constant light quality.

Often, the next step after conducting controlled studies is to introduce pre-counted seeds into field plots. Section 7.3 outlines some methods for how to study germination under field conditions. Treatments may consist of canopies comprising different species (Burton and Bazzaz 1991) or different degrees of canopy opening to test, for example, the effects of full shade, partial shade, and full sun on direct seeding spots (Smith and Clark 1960; Burton 1997). Canopy cover effects can be measured within a stand, or at different distances from a stand edge (Burton 1996; Coates and Burton [1998]). These two conditions evaluate slightly different aspects of the influence of light and shade; different canopy thinning levels alter the size and duration of sunflecks, while plots placed at different distances from a stand edge differ primarily in the daily duration of uninterrupted direct sunlight.

When studying canopy treatment effects on seed production, seed rain density, or seed dispersal, differences in the seed supply are of greater importance than differences in the amount or quality of light. Seed trap arrays, either randomly distributed under different canopy treatments or in transects away from a stand edge, are one of the most common methods for monitoring the influence of forest canopies on seed supply (Figure 4.5 and Section 4.4).

8.3 Effects of Seedbed Manipulation

The success of natural regeneration or direct seeding depends, in part, on how well seedbed environments meet the requirements for seed germination and seedling establishment. The suitability of seedbeds is determined by many factors, including their thickness, water-holding capacity, hydraulic continuity, thermal properties, and chemistry. These factors are so inextricably linked that ecological and silvicultural researchers have found it useful to characterize the seedbed as a single (though complex) environmental factor or metafactor, which is usually described as a categorical variable.

8.3.1 Seedbed preferences

Microsites are sometimes evaluated as a way to compare the suitability of seedbeds. In the following discussion, the term *seedbed* could include many different types of microsites for seed germination, such as different elevations or aspects of a mound or berm, or different degrees of mixing of the forest floor with the mineral soil.

Typical seedbeds encountered in managed forests include mineral soil (compacted or not compacted), forest floor (possibly divided into undecomposed litter and decomposed humus layers), logs or rotting wood, and mats of mosses or lichens. Other substrates, such as bare rock, standing water, or undecomposed logging slash, are never considered as potential seedbeds. Combinations of materials are sometimes recognized, such as moss on rotting wood versus moss on mineral soil, or different (measured) thicknesses of forest floor over mineral soil or rock. Most of these materials are considered to be distinct when consumed or scorched by fire.

Because of wide differences in physical characteristics, temperatures, and the availability of water and mineral nutrients, seedling establishment varies greatly in different natural seedbeds (see Table 8.1 and example in Section 8.3.4). Mineral soil is a good seedbed because of its high infiltration capacity, adequate aeration, and close contact between soil particles and seeds (Kramer and Kozlowski 1979). Although mineral soils warm earlier and attain higher temperatures in the spring, in bright sunlight the surface

TABLE 8.1 *Comparative seedbed suitability of some northwestern tree species (from Minore 1979). Species in the upper groups are better suited to the seedbed than those in lower groups. Data are insufficient for species comparisons within the groups.*

Organic seedbeds		Mineral soil seedbeds		Burned seedbeds
Coastal	**Interior**	**Coastal**	**Interior**	
Tsuga heterophylla	*Picea glauca, Pseudotsuga menziesii*	*Alnus rubra*	*Larix occidentalis, Picea engelmannii*	*Pseudotsuga menziesii, Abies grandis, Pinus ponderosa*
Thuja plicata	*Abies lasiocarpa, Pinus contorta*	*Picea sitchensis*	*Pinus contorta, Abies lasiocarpa*	*Tsuga heterophylla, Larix occidentalis, Picea engelmannii, Pinus contorta*
Picea sitchensis	*Larix occidentalis, Picea engelmannii*	*Thuja plicata*	*Pseudotsuga menziesii, Picea glauca*	*Thuja plicata, Pinus monticola, Abies lasiocarpa*
Alnus rubra		*Tsuga heterophylla*		

temperature of mineral soils and burned materials can become so high as to be lethal to germinating seeds. However, when moisture is adequate, most native British Columbia hardwoods and conifers germinate best on mineral soil seedbeds.

Litter and duff are less suitable than mineral soil because they warm slowly, inhibit root penetration, prevent good seed–mineral soil contact, dry rapidly, and shade small seedlings. Sphagnum moss often is a suitable seedbed because of its high water-holding capacity, but it may subsequently smother young seedlings. Decayed wood is also an excellent natural seedbed for seeds of forest trees, probably because of its capacity for water retention (Knapp and Smith 1982; Harmon et al. 1989). However, although forest floor (or duff) and moss layers may be suitably moist during the spring, they often dry out faster than mineral soil and rotting wood. Therefore, organic materials usually form better seedbeds when they are under partial shade.

Duff seedbeds are better tolerated by large-seeded species because these species can use their stored reserves to subsidize radicle penetration to the mineral soil. Thus, in boreal and subalpine forests, an organic forest floor is an acceptable substrate for true fir (*Abies* spp.) seeds (Alexander et al. 1984), but would not be suitable for the smaller seeds of spruce (*Picea* spp.) (Noble and Ronco 1978; Klein et al. 1991). Some species can establish on both mineral soil and on moss and duff. If sufficient moisture is present, amabilis fir, subalpine fir, tamarack, Engelmann spruce, black spruce, Sitka spruce, Douglas-fir, western hemlock, and mountain hemlock have been found to germinate well on organic and inorganic substrates (Fowells [compiler] 1965; McCaughey 1993).

For some species, the properties of organic substrates preclude seedling establishment. Western redcedar, because of its small seeds, germinates poorly on duff (Fowells [compiler] 1965). Similarly, mineral soil and rotten logs are best for the germination and initial establishment of the very small seeds of paper birch. Newly germinated seedlings are extremely fragile; a paper birch seed that germinates on a fallen hardwood leaf cannot push its radicle through to the moist soil, and if it germinates under a leaf, the tiny seedling is almost always cut off from the light (Hutnik 1954).

The relatively large seeds of bigleaf maple and Garry oak have little problem penetrating leaf litter and organic substrates. Under field conditions, bigleaf maple germination often occurs on relatively undisturbed seedbeds in association with leaf litter and other organic substrates (Tappeiner and Zasada 1993). The best natural seedbed for Garry oak is moist soil covered with 2 cm or more of leaf litter. After

germination, the radicle quickly penetrates deep into the moist mineral soil (Fowells [compiler] 1965). In undisturbed forest, white spruce and western hemlock seedlings often are found on decayed wood, which has several advantages over most other natural seedbeds. For these small seeds, decayed wood usually provides more moisture, less chance of smothering conditions, and freedom from damping-off (Fowells [compiler] 1965).

Very wet conditions are required by western white pine and ponderosa pine seeds for germination and seedling survival, and they are thus restricted more by water availability than by a specific substrate (Fowells [compiler] 1965). Delayed germination and the inability of Rocky Mountain juniper to establish on dry sites probably account for its generally sparse natural regeneration. Juniper seedlings are found most often in the moist soil of rocky crevices and in canyons near perennial water (Fowells [compiler] 1965). Under natural conditions, poplar and willow seeds require a steady supply of water during germination and early seedling development. Seeds of both species can germinate while floating in water or when fully submerged (Wyckoff and Zasada [1998]; Zasada et al. [1998]). The high water requirements of these species is primarily due to their unique pattern of epigeal germination (Figure 7.5). In both poplars and willows, the radicle does not emerge immediately, but instead, a ring of fine hypocotyl hairs (the coronet) performs the dual function of water absorption and initial attachment of seeds to the substrate. Seedlings often fail to survive because the root hairs dry too quickly or fail to securely attach seeds to the soil.

Seedbeds can be manipulated to favour establishment by certain species. The rapid growth of hardwoods may pose serious competition problems for conifers. Red alder, which establishes quickly in full sunlight on exposed mineral soil, is an aggressive pioneer of avalanche paths, road cuts, log landings, skid trails, or other areas where mineral soil has been freshly exposed to seedfall. To exclude red alder, some forest managers try to reduce alder seed supplies by removing seed trees in the vicinity, and by disturbing the site as little as possible to avoid creating favourable seedbed conditions for red alder (Lousier and Bancroft 1990).

8.3.2 Site preparation

Mechanical site preparation equipment was first used to expose mineral soil for natural regeneration, not for planting (Smith 1986). Mechanical site preparation not only modifies the relative abundance of seedbed materials by increasing the exposure of mineral soil, but also creates new microsites with raised or depressed elevations or changes in aspect. Site preparation operations may remove, mix, or invert the organic layer and upper soil horizon to create suitable microsites for seedling establishment (often called planting spots) (McMinn and Hedin 1990). Some seedbed scarification methods, such as dragging anchor chains or shark-fin barrels, remain common means to enhance natural regeneration of lodgepole pine and western larch.

Prescribed burning is detrimental to natural regeneration of conifers because conifer seeds are generally on or close to the surface of the forest floor, and are therefore vulnerable to even a low-severity fire. However, fire creates seedbeds with better heat-holding capacity and seed-substrate contact, and if some residual cover and seed trees remain in the surrounding area, seeds dispersed after a fire are more likely to establish greater numbers of seedlings. Prescribed burning releases nutrients more rapidly than through usual biological degradation processes; however, depending on site factors, these may or may not be available to seedlings (Chapter 22 in Pritchett 1979).

8.3.3 Suggested questions for seedbed studies

Field seedbed research generally focuses on comparing different seedbed attributes (type, amount, or response to manipulation) and how they affect tree establishment. Substrate manipulation does not usually affect seed availability on the ground, because natural seedfall is primarily related to the overstorey. Seed banks and seed predators could, however, be influenced by site preparation. Site preparation can influence the microtopography of the seedbed and, for very small seeds such as willows and poplars, this can be critical. A heterogeneous seedbed comprised of particles of litter or soil prominences can strand these seeds on rapidly drying surfaces where either seeds do not germinate, or root hair growth is insufficient to make firm contact with the water-supplying substrate (McDonough 1985).

Typical research questions associated with seedbed manipulation include the following:

- Which seedbed type is best for conifer or hardwood germination and establishment?
- Is there a critical thickness of forest floor material above which germination or early seedling survival is inhibited?
- Does site preparation (e.g., broadcast slash burning, mechanical site preparation) stimulate or inhibit germination—from the seed bank? —of fresh seeds deposited on the ground surface?
- Is seedling recruitment density significantly greater with or without seedbed scarification (e.g., dragging anchor chains)?
- Is tree seed germination more successful on untreated forest floor or in scarified patches with exposed mineral soil?
- What is the seed-to-seedling ratio under alternative site preparation treatments?
- Will seedbeds suffer greater predation in forested or in open conditions?
- Does one tree species germinate and/or establish better than another under a particular site preparation treatment (e.g., a broadcast slashburn)?
- Does the influence of seedbeds on tree seed germination depend on canopy conditions? For example, is mineral soil significantly better than organic substrates for germination under all canopy conditions, or just in open areas?
- How quickly do seedbeds "deteriorate" and how does this affect seed-to-seedling ratios?
- Can seedbeds be manipulated to enhance (or reduce) hardwood regeneration relative to that of conifers?

8.3.4 Methods for seedbed research

Some seedbed studies have used sieved materials in pots placed in growth chambers or greenhouses (Minore 1972; Ahlgren and Ahlgren 1981). Such studies may be used to demonstrate allelopathic effects (such as Brown 1967; Yoder-Williams and Parker 1987), but do not replicate the thermal and moisture dynamics affecting seed germination in forest seedbeds. Pots or trays containing experimental seedbeds, even when used in the field (though less so if buried in the ground), dry more rapidly along the edges, or retain moisture at the base. Seedbed trials are best conducted within frames (cylindrical or rectangular) that allow full hydraulic contact of the test material with the underlying soil. At the very least, containers should be placed flush with the ground surface.

Another approach is to use removable germination containers filled with intact substrate from the study site. Haeussler et al. (1995) constructed cylinders (8 cm diameter, 5 cm high) from PVC pipe and glued fine nylon mesh to the base. A core of forest floor or mineral soil was removed from the plot and placed intact into a container. The container was replaced into the core hole with its upper rim protruding 1 cm. Seeds were sown into each container, and a protective cage to exclude predators (60 mm plastic mesh measuring 8 × 8 cm, 6 cm high) was staked over each germination container.

The establishment of tree seedlings on a variety of substrates has been investigated for many ecosystems (Fisher 1935; Minore 1972). While controlled and replicated experimental treatments are preferable, the same information can be inferred from sample surveys comparing the observed abundance of seedlings found on different seedbeds with the expected abundance of seedlings on those seedbeds (Christy and Mack 1984; Geier-Hayes 1987). Only young or small seedlings (less than 5 years old) should be counted because the seedbeds from which they are derived can deteriorate over time. This approach is discussed in the following example and in Table 8.2a and Table 8.2b.

EXAMPLE

(P. Burton and N. Daintith, unpublished data)

Objective. To determine whether naturally regenerated subalpine fir, interior spruce, and Douglas-fir seedlings exhibit any association with different seedbeds within a partially cut Douglas-fir stand.

Hypothesis. If there is no difference between substrates for germination and establishment of tree seedlings, then the abundance of seedlings found on each substrate will be proportional to the abundance of that substrate.

Method. Seedbed abundance in an interior Douglas-fir stand northeast of Williams Lake, B.C., was measured by estimating percent cover along line transects spaced at regular intervals (Table 8.2a). All

TABLE 8.2(a) *The relative abundance of seedbed substrates in an interior Douglas-fir stand (P. Burton and N. Daintith 1994, unpublished data)*

	Mineral soil	Disturbed duff/moss	Undisturbed duff/moss	Rotten wood
seedbed as % of total area	2.74	11.88	66.73	18.65

TABLE 8.2(b) *Expected and observed seedling association with four forest floor substrates in an interior Douglas-fir stand*

	Mineral soil		Disturbed duff/moss		Undisturbed duff/moss		Rotten wood		Total observed seedlings	Chi-square value[a]
	Exp	Ob	Exp	Ob	Exp	Ob	Exp	Ob		
Subalpine fir	1.2056	0	5.2272	2	29.3612	22	8.2060	20	44	21.99
Interior spruce	0.5206	0	2.2572	1	12.6787	5	3.5435	13	19	31.11
Douglas-fir	3.7264	0	16.1568	1	90.7528	42	25.3640	93	136	224.49

Exp = expected number of seedlings (null hypothesis);
Ob = observed number of seedlings (survey data).
[a] See chi-square calculations below.

Calculations to determine the goodness-of-fit for different seedling and seedbed associations

Subalpine fir:

Chi-square = $\sum$ (observed - expected)^2 / expected
= $(0\text{-}1.2056)^2 / 1.2056 + (2\text{-}5.2272)^2 / 5.2272 + (22\text{-}29.3612)^2 / 29.3612 + (20\text{-}8.2060)^2 / 8.2060$
= 1.2056 + 1.9924 + 1.8455 + 16.9508
= 21.99 (exceeds the critical value 7.815)

Interior spruce:

Chi-square = $\sum$ (observed - expected)^2 / expected
= $(0\text{-}0.5206)^2 / 0.5206 + (1\text{-}2.2572)^2 / 2.2572 + (5\text{-}12.6787)^2 / 12.6787 + (13\text{-}3.5435)^2 / 3.5435$
= 0.5206 + 0.7002 + 4.6505 + 25.2365
= 31.11 (exceeds the critical value 7.815)

Douglas-fir:

Chi-square = $\sum$ (observed - expected)^2 / expected
= $(0\text{-}3.7264)^2 / 3.7264 + (1\text{-}16.1568)^2 / 16.1568 + (42\text{-}90.7528)^2 / 90.7528 + (93\text{-}25.3640)^2 /25.3640$
= 3.7264 + 14.2187 + 26.1902 + 180.3591
= 224.4944 (exceeds the critical value 7.815)

the seedlings encountered in separate regeneration survey plots were classified according to the seedbed in which they were found (*observed values*, Table 8.2b). The *expected value* in each cell (seedbed/species category) is derived from the percentage of the seedbed area multiplied by the total number of seedlings of the species encountered (e.g., 2.74% mineral soil × 44 subalpine fir seedlings = 1.2056).

Chi-square values are calculated separately for each species based upon *observed* and *expected* values, as shown below. Values are calculated for each seedling and seedbed association, then summed to create a chi-square test statistic for the species.

If the chi-square test statistic for a species exceeds the critical value (determined from published tables of the chi-square distribution), then seedbeds—in general—have a significant effect on seedling establishment. In addition, if one value of the sum exceeds the critical value, then that individual value is significantly different.

Null hypothesis. In Table 8.2a, the null hypothesis of random association of seedlings and seedbeds is rejected for $\alpha = 0.05$ and degrees of freedom = 3 (number of seedbed categories, minus one).

Results:

1. The calculated chi-square test statistic exceeded the critical value (7.815) for each species, indicating that the observed seedling/seedbed associations were non-random.
2. A comparison of chi-square test statistics for different seedbeds shows that values contributed by rotten wood exceeded the critical value (7.815) for all three species: subalpine fir (16.9508), interior spruce (25.2365), Douglas-fir (180.3591).
3. In addition, for Douglas-fir, the calculated chi-square test statistic exceeded the critical value (7.815) for disturbed duff/moss (14.2187) and for undisturbed duff/moss (26.1902). Therefore, the lack of seedling establishment on those seedbeds could not be attributed merely to chance.

Conclusions:

Calculated chi-square test statistics for all three tree species confirmed that different seedbeds within a partially cut interior Douglas-fir stand significantly affected seedling establishment.

Comparisons of expected and observed seedling and seedbed associations indicate that seedlings of all three species were found more frequently on rotten wood, and that Douglas-fir seedlings did not establish well on duff/moss substrates. It is not known whether Douglas-fir seeds were unable to germinate on moss/duff substrates, or whether the seedlings emerged, but did not survive.

Cautions on use of the chi-square statistic:

The use of the chi-square test statistic is conditional upon achieving expected values greater than 5.0. An expected value greater than 5.0 must be obtained for the test statistic to be chi-square distributed (Lesperance 1996). In this example, all of the expected values for mineral substrates were less than 5.0, as were the expected values for interior spruce on disturbed duff/moss and rotten wood.

If this situation occurs, there are several alternatives to employ:

1. Increase the sample size. To calculate the sample size required, divide the expected value needed (5.0) by the percentage of the least abundant substrate. In this example, mineral soil represents 2.74% of the total seedbed area. Thus, the minimum number of seedlings required for each species would be 5.0 ÷ 0.0274 = 182.48 seedlings.
2. Combine the data in different columns. Observations for mineral soil could be combined with the observations for disturbed duff/moss or rotten wood. In this case, combining data is probably not a viable option because mineral soil is very different from the other substrates.
3. Rather than assume the chi-square distribution, use a random procedure to determine the empirical distribution. Refer to Manly (1997) for further information on this topic.

Inferring substrate suitability from a one-time survey (as in the example above) may be adequate for some purposes, but understanding the actual constraints to natural regeneration requires establishing controlled treatment plots and evaluating tree seedling emergence and survival over time. In this way, the effects of seed rain, seed predation, seed germination, and germinant survival may be evaluated separately.

For example, seeds may germinate in a wide range of microsites, but may fail to survive as a result of drought conditions over the summer (Potts 1985; Burton 1997). Better-than-average survival is often noted in cohorts of seedlings that germinate earlier, rather than later, in the spring (Zasada et al. 1983). Low seedling densities may not be due to the seedbed, but rather to the limited availability of seeds, the seed-shedding behaviour of associated vegetation, or local activity and habitat preferences of seed predators. Conversely, high numbers of seedlings in a particular microsite may be due to the capture of seeds drifting on a crusted snow surface, to microsites of exposed mineral soil, or to unusually abundant soil water during the growing season (Matlack 1989). If the factors (and their interactions) promoting good regeneration are not understood, site preparation methods cannot be successfully applied to other locations.

8.4 Combined Studies

Studies of seed germination and seedling establishment can be combined with seed rain monitoring to select the optimal width for patch cuts and strip cuts for natural regeneration (see Noble and Ronco 1978; McDonald and Abbott 1994). By coupling seed rain with seedling survival, estimates can be made of the seed-to-seedling ratios required to establish a single seedling that survives to a given age (e.g., 5 years) (see Alexander 1986; Walker et al. 1986).

Seed-to-seedling estimates help us understand the dynamics of major factors influencing successful reforestation of a site. Even with good seed supplies and high emergence, seedling survival can differ significantly among sites and seedbeds, in different years, and between clearcut and forest conditions. For example, in the first growing season, heat and drought accounted for 60% of red alder seedling mortality in clearcuts compared to only 5% of red alder mortality in forests (Haeussler et al. 1995). Later other factors became more important. During the first winter, soil erosion, frost heaving, and freezing together caused over 60% of mortality in clearcuts; during the second growing season, crushing under litter or vegetation (40%) was the primary mortality factor.

Because young seedlings usually require some protection during the first growing season, the interactions between site preparation and shade treatments are often evaluated. For example, Alexander (1986) found that germination and survival of Engelmann spruce in Colorado was poorer on sites that were left unscarified and/or unshaded. Cain (1991) noted that seedbed preparation resulted in better survival of understorey loblolly (*Pinus taeda*) and shortleaf pine (*Pinus echinata*); however, the chemical removal of hardwoods in the canopy (with or without seedbed scarification) was even more important than seedbed treatments in promoting pine survival.

The Engelmann Spruce–Subalpine Fir (ESSF) biogeoclimatic zone is one of the most extensive forest zones in British Columbia, but it is also one the province's most severe climates for forest growth. To determine the best conditions in this area for the natural regeneration of subalpine fir and Engelmann spruce, germination plots received a number of seeding and silvicultural treatments: screefed and seeded (DS), screefed and not seeded (DN), undisturbed and seeded (US), or undisturbed and not seeded (UN) (Jull et al. 1996). Disturbed forest floor, created either by screefing or logging disturbance, dramatically improved the germination of both spruce and subalpine fir seeds (Figure 8.5). Direct seeding of the plots with undisturbed forest floor only slightly improved the total number of germinants relative to unseeded plots. Very small numbers of germinants were observed in clearcut areas when additional seeds were not supplied, even though 1993 (the year in which the study was conducted) was a year of relatively high seed production in the surrounding stands. These results indicate that natural seed supplies cannot be relied on for the reforestation of clearcuts in this area.

8.5 Summary

Seeds, by virtue of their small size, respond to the environmental conditions prevailing within a relatively small microsite. Much of the research and documentation of seed germination ecology under natural conditions thus involves the classification, modification, or monitoring of microsites and the

behaviour of seeds and seedlings within them. Farmer (1997) provides a useful summary of forest microsites as regeneration niches, and how these microsites might be studied and manipulated.

Even the simplest field investigations of seedbed and canopy effects (whether natural or manipulated) constitute a challenging exercise in ecosystem ecology. The potential of a site to support natural regeneration or successful regeneration by direct seeding cannot be evaluated by a single factor isolated from other environmental or site variables. Even though canopy and seedbed influences are, in themselves, complex meta-factors, careful consideration must also be given to a range of other influences, including local climate, the silvics of different tree species, the microsite attributes of vegetation and soil, and many of the other factors that comprise the physical and biological matrix upon which successful natural regeneration depends.

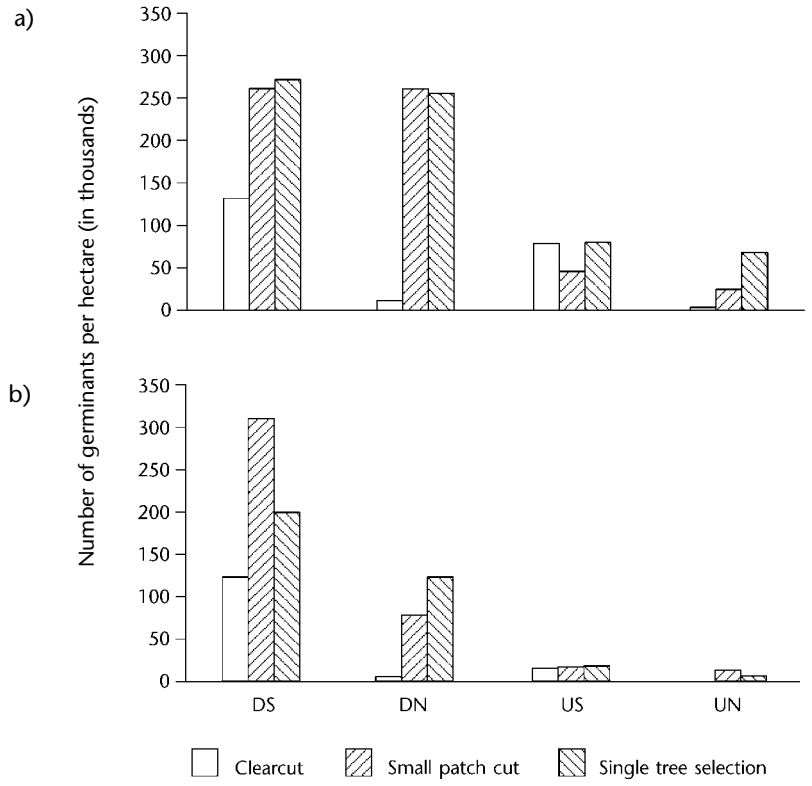


FIGURE 8.5 *Number of (a) subalpine fir and (b) Engelmann spruce germinants per hectare within three silvicultural treatments at the Lucille Mountain Project, Prince George Forest Region, British Columbia (Jull et al. 1996). Results are shown for treatments illustrated in Figure 8.2a, b, and e. DS = screefed and seeded, DN = screefed and not seeded, US = undisturbed and seeded, UN = undisturbed and not seeded.*

APPENDIX A Tree Species Occurring in British Columbia

Gymnosperms

Scientific name/authority	Common name
Abies amabilis (Dougl. ex Loud.) Forbes	amabilis fir
Abies grandis (Dougl. ex D.Don in Lamb.) Lindl.	grand fir
Abies lasiocarpa (Hook.) Nutt.	subalpine fir
Chamaecyparis nootkatensis (D.Don in Lamb.) Spach	yellow-cedar
Juniperus scopulorum Sarg.	Rocky Mountain juniper
Larix laricina (Du Roi) K.Koch	tamarack
Larix lyallii Parl. in DC.	subalpine larch
Larix occidentalis Nutt.	western larch
Picea engelmannii (Parry ex Engelm.)	Engelmann spruce
Picea glauca (Moench) Voss	white spruce
Picea mariana (P.Mill.) B.S.P.	black spruce
Picea sitchensis (Bong.) Carr.	Sitka spruce
Pinus albicaulis Engelm.	whitebark pine
Pinus banksiana Lamb.	jack pine
Pinus contorta Dougl. ex Loud. var. *contorta*	shore pine
Pinus contorta Dougl. ex Loud. var. *latifolia* Engelm.	lodgepole pine
Pinus flexilis James	limber pine
Pinus monticola Dougl. ex D.Don in Lamb.	western white pine
Pinus ponderosa Dougl. P. & C. Lawson	ponderosa pine
Pseudotsuga menziesii (Mirb.) Franco var. *glauca* (Beissn.) Franco	Rocky Mountain (interior) Douglas-fir
Pseudotsuga menziesii (Mirb.) Franco var. *menziesii*	coastal Douglas-fir
Taxus brevifolia Nutt.	Pacific yew
Thuja plicata Donn ex D.Don in Lamb.	western redcedar
Tsuga heterophylla (Raf.) Sarg.	western hemlock
Tsuga mertensiana (Bong.) Carr.	mountain hemlock

Angiosperms

Scientific name/authority	Common name
Acer macrophyllum Pursh	bigleaf maple
Alnus rubra Bong.	red alder
Arbutus menziesii Pursh	arbutus
Betula papyrifera Marsh.	paper birch
Betula papyrifera var. *neoalaskana* (Sarg.) Raup.	Alaska paper birch
Cornus nuttallii Aud. ex T. & G.	Pacific dogwood
Fraxinus latifolia Benth.	Oregon ash
Malus fusca (Raf.) Schneid.	Pacific crab apple
Populus balsamifera L. ssp. *balsamifera*	balsam poplar
Populus balsamifera L. ssp. *trichocarpa* (T. & G.) Brayshaw	black cottonwood
Populus tremuloides Michx.	trembling aspen
Prunus emarginata (Dougl.) Walp.	bitter cherry
Prunus pensylvanica L.	pin cherry
Quercus garryana Dougl.	Garry oak
Rhamnus purshiana DC.	cascara
Salix amygdaloides Anderss.	peach-leaf willow
Salix bebbiana Sarg.	Bebb's willow
Salix discolor Muhlenb.	pussy willow
Salix exigua Nutt.	sandbar willow
Salix lucida Muhl. ssp. *lasiandra* (Benth.) E. Murray	Pacific willow
Salix scouleriana Barratt ex Hook.	Scouler's willow

Standard species names and codes for British Columbia can be found in both ACCESS 2.0 and EXCEL 4.0 files at the B.C. Ministry of Forests Research Branch FTP site (142.36.141.53) (anonymous login) in the directory /pub/provspp. They are regularly revised and updated.

APPENDIX B Conversion Factors

Imperial units		SI equivalents
Length		
1 chain = 66 feet	=	20.1168 m (exactly), or a 50 or 75 m nylon tape
1 foot	=	0.3048 m (exactly)
1 inch	=	2.54 cm (exactly)
1 mile	=	1.6093 km
1 yard	=	0.9144 m (exactly)
Area		
1 acre	=	0.4047 hectare
1 square foot	=	0.0929 m^2
1 square inch	=	6.4516 cm^2 (exactly)
1 square mile	=	2.5900 km^2
1 square yard	=	0.8361 m^2
Mass		
1 ounce	=	28.3495 g
1 pound	=	453.60 g
1 ton (2,000 lb.)	=	0.9072 t
Volume or Capacity (dry measure)		
1 US bushel = 2150.42 cu. in.	=	approx. 35.24 L
1 UK bushel (liq & dry) = 8 imp gal	=	approx. 36.37 L
1 US peck = (¼ US bushel)	=	8.81 L
1 UK peck = 2 gals. or 8 imp qts.	=	9.0925 L
1 imp gal	=	4.54609 L (exactly)
1 U.S. gal	=	3.78541 L
2.838 US bushels	=	100 L or 1 hectolitre

Approximate conversion factors for light
Daylight, full sun*
950 W m^{-2} = 1.36 cal cm^{-2} min^{-1} ≅ 95,000 lux of this PAR (400–700 nm) is: 1800 µmol photons m^{-2} s^{-1} ≅ 399 W m^{-2} = 0.572 cal cm^{-2} min^{-1} = 42% of total. ∴ 1 W m^{-2} (total) ≅ 1.895 µmol photons m^{-2} s^{-1} (PAR)
Blue sky light*
72 W m^{-2} = 0.103 cal cm^{-2} min^{-1} ≅ 9000 lux of this PAR is: 200 µmol photons m^{-2} s^{-1} ≅ 45 W m^{-2} = 0.065 cal cm^{-2} min^{-1} = 63% of total. ∴ 1 W m^{-2} (total) ≅ 2.778 µmol photons m^{-2} s^{-1} (PAR)

**Data from Coombs et al. (1985).*

Cone and Seed Analysis Companies and Suppliers

For a current list of cone and seed analysis companies and suppliers, please refer to Portlock (compiler, 1996).

Internet Resources

Solar noon estimates
http://www.crhnwscr.noaa.gov/grr/sunlat.htm

B.C. Ministry of Forests Internet resources
homepage: http://www.for.gov.bc.ca/

Glossary of terms: http://www.for.gov.bc.ca/pab/publctns/glossary/glossary.htm

Publication catalogue: http://www.for.gov.bc.ca/hfd/pubs/search/index.htm

Forest Practices Code homepage: http://www.for.gov.bc.ca/tasb/legregs/fpc/fpc.htm

Current species lists: Res. Br. FTP site (142.36.141.53), in directory /pub/provspp.

Research Branch FTP site: address of the Cowichan server is 142.36.141.53 The directory will depend on your enquiry.

Nursery and Seed Operations Branch Homepage http://www.for.gov.bc.ca/nursery/branch.htm

SPAR homepage
http://www.for.gov.bc.ca/nursery/headqtrs/spar.htm

LI-COR (radiometer) homepage
http://www.licor.com

Computer Programs

The computer programs mentioned in the text are familiar to the authors, but this does not imply endorsement or that they are the only programs available for the task. Further information about each program is available from the contacts and references provided below.

Sit (1992a) lists the code of a SAS program for computing tree shadow lengths for different times of day and year.

GLI
Dr. Charles Canham, Institute of Ecosystem Studies, Box R, Millbrook NY 12545-0178 USA
Reference: Canham (1988)

GS+ (Professional Geostatistics for the PC, Version 2.0 (1992).
Gamma Design Software, P.O. Box 201, Plainwell MI 49080 USA

HEMIPHOT
The Tropenbos Foundation, P.O. Box 232, 6700 AE Wageningen, The Netherlands
Reference: ter Steege (1993)

SAS (Statistical Analysis System)
SAS Institute Inc., Box 8000, Cary NC 27512-8000 USA

SiteTools Software
(for estimating site series from species and height)
B.C. Ministry of Forests, Research Branch, P.O. Box 9519, Stn. Prov. Govt., Victoria, BC V8W 9C2

SOLARCALC
Dr. Robin Chazdon, Department of Ecology and Evolutionary Biology, University of Connecticut, Box U-42, Storrs CT 06269-3042 USA
Reference: Chazdon and Field (1987)

Computer Programs *(continued)*

SUNSHINE

Mr. W. Rick Smith, Research Forester, USDA Forest Service, Southern Forest Experiment Station, 701 Loyola Ave., New Orleans LA 70113 USA
Reference: Smith and Somers (1991)

SYSTAT/SYGRAPH

SPSS Inc., 444 North Michigan Avenue, Chicago IL 60611–3962 USA

VENUS

VENUS is a computer data entry and reporting tool for describing site data (soil, mensuration, vegetation), and is based on the FS882 forms available from B.C. Ministry of Forests Sales. The program is available from the B.C. Ministry of Forests Research Branch FTP site (address: 142.36.141.53) in directory / pub/venus. For further information, contact the Ecology Data Analyst (Greg Britton):

B.C. Ministry of Forests, Research Branch, P.O. Box 9519, Stn. Prov. Govt., Victoria, BC V8W 9C2

Other Resources

LI-COR RADIOMETER

LI-COR Inc., 4421 Superior St., P.O. Box 4425 Lincoln NE 68504, USA
Telephone 1-800-447-3576 (U.S. and Canada) or 402-467-3576; FAX: 402-467-2819
email: envsales@env.licor.com

(SPAR) Seedling Planning and Registry System

SPAR is an on-line registry, intended for use by B.C. Ministry of Forests staff, licensee, and nursery staff whose job responsibilities require it. The registry facilitates entering Seedling Requests, managing the Tree Seed Register for seedlots and the Cutting Registry for cutting lots, and monitoring Seedling and other Cone and Seed Service Requests. Services also include electronic access for all Ministry and non-Ministry clients and data entry of seedling request and seedlot provenance information at the forest district level.

Internet: SPAR homepage at http://www.for.gov.bc.ca/nursery/headqtrs/spar.htm

Biogeoclimatic Ecosystem Classification System

The biogeoclimatic ecosystem classification (BEC) is a hierarchical land classification system used in British Columbia that delineates ecological units based on vegetation, soils, and climate. BEC information for specific regions in British Columbia can be found in the following publications:

Forest region	References
Cariboo	Steen et al. [1997]
Kamloops	Lloyd et al. (1990)
Nelson	Braumandl and Curran (1992)
Prince George	DeLong (1996a) DeLong (1996b) Delong et al. (1993) Delong et al. (1994) MacKinnon et al. (1990) Meidinger et al. (1996)
Prince Rupert	Banner et al. (1993)
Vancouver	Green and Klinka (1994)

The BEC climate summary database is not yet available on the Internet. For information, contact David Spittlehouse, Research Branch, B.C. Ministry of Forests, P.O. Box 9519, Stn. Prov. Govt., Victoria, BC V8W 9C2

Forest Practices Code Guidebooks

Information about the B.C. Forest Practices Code and a list of current guidebooks is available on the Forest Practices Code homepage. Guidebooks can be ordered through the Guidebook page or by telephone, fax, or mail from the address below.

Forest Practices Code homepage: http://www.for.gov.bc.ca/tasb/legregs/fpc/fpc.htm

Forest Practices Code Guidebooks, Public Affairs Branch, B.C. Ministry of Forests, P.O. Box 9517 Stn. Prov. Govt., Victoria, BC V8W 9C2
Telephone: 1-800-994-5899 or 250-387-7964
FAX: 250-387-7009

GLOSSARY

Felix que potuit rerum cognoscere causes.
—Happy he who can understand the causes of things.
(Virgil)

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abscission The separation of an appendage (petiole, fruit stalk, etc.) as a result of the programmed death of a specialized zone of cells (the abscission layer) found at the base of the appendage.

achene A dry, indehiscent (non-opening) one-seeded fruit (e.g., fruit of *Betula*).

accuracy The closeness of a set of estimates to the true population parameter, considered together with how closely they are grouped together (their precision). Compare *precision*.

acorn The one-seeded fruit of oaks; consists of a cup-like base and the nut (e.g., fruit of *Quercus garryana*).

adjusted coefficient of determination See *coefficient of determination*.

allometric Refers to the study and measurement of the growth of part of an organism relative to the whole.

analysis of covariance (ANCOVA) A statistical tool that combines both ANOVA and regression. The treatment means of the dependent variable are adjusted by using a covariate which controls error and increases precision. See ANOVA, *regression*, *precision*.

analysis of variance (ANOVA) A statistical tool used to analyze differences observed in the means of treated samples, to determine whether the differences in the means are due to the treatment or to random variation in the population.

anemometer An instrument for measuring wind speed, which may give direct or recorded readings.

angiosperms Flowering plants, distinguished from gymnosperms by having the ovules enclosed within the ovary; after fertilization the ovary becomes a fruit, enclosing one or more seeds. Compare *gymnosperms*.

aril Exterior covering or appendage that develops after fertilization as an outgrowth from the point of attachment of the ovule (e.g., fleshy fruit of yew containing a single seed).

artificial regeneration Establishing a new forest by planting seedlings or by direct seeding. Compare *natural regeneration*.

aspect The direction toward which a slope faces, expressed in degrees azimuth (clockwise from north), or categorized according to 4 (N, S, E, or W), 8, or 16 compass points.

auger A tool used to bore into wood or soil to retrieve a cylindrical sample or core.

autocorrelation The correlation between a point in a set and other points within the same set.

berry A pulpy fruit developed from a single pistil and containing one or more immersed seeds, but no true stone (e.g., fruit of *Arbutus menziesii*).

biogeoclimatic ecosystem classification (BEC) A hierarchical land classification system used in British

Columbia that delineates ecological units based on vegetation, soils, and climate.

biogeoclimatic site series within the BEC system all sites capable of producing the same mature or climax plant communities within a biogeoclimatic subzone or variant. Site series are described by the site and soil conditions as well as the vegetation community.

biogeoclimatic subzone geographic areas influenced by one regional climate. Subzones are divided into variants and site series.

biogeoclimatic variant subzones are sometimes further divided into areas called variants which reflect variations in climate (e.g., drier, wetter, snowier, warmer, or colder) within the subzone.

biogeoclimatic zone within the BEC system, generalized units representing extensive areas of broad, homogeneous macroclimates. Zones are divided into subzones.

biological diversity (biodiversity) The diversity of plants, animals, and other living organisms in all their forms and levels of organization, including genes, species, ecosystems, and the evolutionary and functional processes that link them.

Bonferroni technique A statistical method for making several non-independent pairwise comparisons, usually performed after ANOVA.

bract In gymnosperms, a modified leaf that extends underneath a scale in a female cone.

breast height age The number of annual growth rings measured on a tree at breast height, 1.3 m above high side ground level. See also *dbh*.

canopy (1) The cover of branches and foliage formed by tree crowns. (2) The branches and foliage of any vegetation.

canopy bank All seeds retained in cones or fruits on the tree, as opposed to seeds being retained in the soil. Compare *soil seed bank*.

capsule A dry, many-seeded fruit composed of two or more fused carpels that split at maturity to release their seeds (e.g., fruit of *Alnus, Betula, Populus)*.

categorical variable See *variable*.

catkin In gymnosperms, a male strobilus which produces pollen. In angiosperms, a spike-like inflorescence, usually pendulous, of unisexual flowers (either staminate or pistillate) (e.g., *Alnus, Betula, Populus, Salix*).

central tendency A measure of the "middle" of a distribution. Common measures of central tendency are mean (the average), the median (the middle value of an ordered set), and the mode (the value with highest frequency).

chi-square test A statistical test for analyzing categorical variables measured for two or more populations. See *variable - categorical.*

chord An aeronautics term: an imaginary straight line between the leading and trailing edges of an airfoil.

chromosome The genetic material of organisms; composed of DNA and proteins.

clearcut (n. or adj.) An area of forest land from which all trees have been harvested. **clear-cut** (v.) A timber harvesting method and an even-aged silvicultural system in which all trees (typically >3 m tall) are removed to maximize the recovery of fibre and to provide growing space for the next crop.

closed canopy Describes a stand in which the crowns of the main level of trees forming the canopy are touching and intermingled so that light cannot reach the forest floor directly. See *canopy*.

codominant In stands with a closed canopy, those trees whose crowns form the general level of the canopy and receive full light from above, but comparatively little from the sides. In young stands, those trees with above-average height growth. See *canopy*, *crown class.*

coefficient of determination (r^2) A statistic that assesses how clearly a regression model describes the

relationship between the dependent and independent variables. Adjusted r^2 is the coefficient of determination adjusted by the model degrees of freedom, and is more appropriate than r^2 for comparing several models using the same data.

coefficient of variation (CV) A measure of variation relative to the mean; the ratio of standard deviation to the mean.

cohort A group of organisms of more or less the same age (e.g., all seedlings that germinated in the month of May).

cone The dry multiple fruit of conifers. A female cone consists of a central axis supporting scales which bear naked seeds. A male cone consists of a central axis supporting spirally arranged microsporophylls bearing pollen sacs that contain pollen grains. Syn. *strobilus*. See *conelet*, *microsporophyll*.

conelet In gymnosperms, the immature stages of development of a female flower following pollination. See *cone*, *female flower*.

confidence interval A range of possible values above and below an estimate of some population parameter, expressing the likelihood (e.g., 95%) that the true value lies between the bounds of that range. The confidence level is the probability that a confidence interval will enclose the true value of the parameter; 1 – confidence level = level of significance. Compare *level of significance*.

continuous (of data or a variable). See *variable*.

coppice A silvicultural system that takes advantage of the tendency of some trees to produce many shoots when the main stem is removed and the root system is left intact.

cotyledon The first leaf produced by the embryo of a seed plant. In conifers, cotyledons appear needle-like.

cover The vertical projection of the crown or stem of a plant onto the ground surface; usually expressed as a percentage of the total ground area being considered.

crown The live branches and foliage of a tree.

crown class A group of trees in a forest having crowns of similar development and occupying a similar position in the canopy. See *canopy, dominant*, *codominant*.

cutting test A method to determine seed maturity; the seed is bisected longitudinally and the morphological development of the embryo and the storage tissue are assessed.

datalogger A portable, rugged simple computer, typically used in the field to automatically record data from environmental sensors over a period of time. The data can then be transferred to a computer and returned to the lab for analysis.

dbh Diameter at breast height; a standard forestry measurement used to indicate stem diameter 1.3 m above ground level.

detection limit The resolution or finest distinction that can be measured with a particular instrument or methodology.

dioecious (Literally *two houses*). Describes plants in which the male (staminate) and female (pistillate) flowers are borne on different plants. Compare *monoecious*.

direct count A method for determining the number and species of seeds found in a soil seed bank by separating seeds from the soil, then counting and identifying them directly. Compare *sample germination*. See *elutriation*.

direct seeding The practice of sowing seeds in or on the soil, rather than planting seedlings to reforest a harvested area. See *artificial regeneration*.

discrete See *variable*.

dispersal Movement of individuals away from a source, as in the spread of seeds away from a parent plant.

dispersal curve The frequency distribution of dispersed seed versus the distance that seeds are found from the seed source.

dispersion The spatial arrangement of objects, often described as random, clumped, or regular.

dominant Trees with crowns extending above the general level of the canopy and receiving full light from above and partly from the side; taller than the average trees in the stand and with well-developed crowns. See *canopy*, *crown class, codominant*.

dormancy Physical or physiological condition of a viable seed that prevents germination even in the presence of otherwise favourable germination conditions.

drupe Fleshy indehiscent fruit, usually one-seeded, containing a seed enclosed in a hard, bony endocarp (pericarp), (e.g., fruit of *Cornus, Prunus*). S*yn.* stone fruit.

elutriation The process of separating soil and particles from seeds. See *root elutriator.*

embryo The rudimentary plant within the seed; that part of a seed that develops from the union of the egg cell and sperm cell, which after germination becomes the young plant.

emergence (1) Protrusion of the radicle through the seed coat, or (2) under nursery or field conditions, protrusion of the hypocotyl and cotyledon above the soil surface.

emittance The radiant flux emitted per unit area of a surface. Compare *irradiance*.

empty seed A seed that does not contain all the tissues essential for germination. Compare *filled seed*.

endosperm Nutritive tissue (3N) of an angiosperm seed, which surrounds and nourishes the embryo. Compare *megagametophyte*.

epicotyl That portion of the seedling stem above the cotyledons.

epigeal Seed germination in which there is considerable elongation of the hypocotyl so that the cotyledons are raised above the surface of the ground to form the first green leaves of the plant. Compare *hypogeal*.

even-aged Describes a forest, stand, or forest type in which relatively small age differences (10–20 years) exist between individual trees which could be considered members of a single *cohort*. Compare *uneven-aged.*

excised embryo test: A quick test for evaluating the growth potential of an embryo that has been removed from the seed.

exclosure A cage placed around a field plot to exclude predators of seeds or seedlings, or other animal activity.

experimental unit. The smallest collection of the experimental material to which one level of a factor or some combination of factor levels is applied.

factor Some influence that is thought to cause a response (e.g., soil moisture or soil type may affect germination rate). Often used as a synonym for "treatment." A factor may be either fixed or random. The levels of a fixed factor (e.g., soil moisture) are chosen by the experimenter, and replication of the experiment would involve those same factor levels. The levels of a random factor (e.g., soil type) are chosen in a random manner from the population of all possible levels, and replication of the experiment would (possibly) involve a new random set of levels.

female flowers (1) The female strobili of conifers before and during pollination. (2) The flowers of angiosperms that contain female structures (ovary and style), but not male structures. See *cone, conelet, strobilus*.

fertilization Penetration of a pollen tube into the ovule; the male sperm nucleus is discharged into the ovule to unite with the egg nucleus.

filled seed A seed that contains both storage tissue and an embryo, as opposed to being empty or partially empty. Compare *empty seed*.

fixed factor See *factor*.

flora The plant life characteristic of a particular geographic area.

frugivore (adj. **frugivorous**): An animal that eats fruit.

gamma radiation High-energy electromagnetic radiation emitted by excited atomic nuclei passing to a lower excitation state; a useful tag for retrieving seeds. See *radioactive*.

gap The space left in the canopy when one or more trees die or are removed. See *canopy*.

genotype The hereditary constitution of an individual organism, which may or may not be expressed as observable features. Compare *phenotype*.

geostatistics A branch of applied statistics that focuses on the detection, modelling, and estimation of spatial patterns.

germinant A young seedling, just after emergence from the seed, but before full establishment as an independent plant.

germination Resumption of active growth in the embryo, which results in emergence of the embryo from the seed and development of the embryo into an independent plant.

germination pecentage An expression of how many seeds germinated as a percentage of the total number of seeds sown; = number of seeds germinated ÷ number of seeds sown × 100.

germination rate (R50) The number of days it takes for 50% of the total number of sown seeds to germinate.

germination speed (R50') The number of days it takes for 50% of the germinating seeds to germinate.

germination value (GV) An expression that combines the speed and completeness of germination into a single number; GV = peak value (PV) × mean daily germination (MDG). PV = maximum quotient obtained by dividing the number of accumulated daily germination by the corresponding number of days. MDG = total germination divided by the number of days in the test. Compare *germination rate, germination speed*.

global radiation See *solar radiation*.

group selection system A harvesting and silvicultural system designed to regenerate an uneven-aged stand by removing trees in small groups. See *silvicultural system*, *uneven-aged*.

growing degree-days (GDD) A cumulative sum of the degrees of temperature above a threshold (generally 5°C) counted on each day that the daily mean temperature exceeds that threshold.

gymnosperms Conifers and their allies; distinguished from angiosperms by having unprotected ovules (not enclosed in a fruit). Compare *angiosperms*.

half-face The cut surface of one side of a cone that has been bisected longitudinally.

hybrid The offspring produced by crossing individuals of different species or unrelated genetic lines. Usually refers to crossing of two true-breeding individuals (homozygous) with different forms of a trait (e.g., green or yellow seeds); the offspring are heterozygous hybrids.

hydration (of seeds) Uptake of water by seed tissues.

hypocotyl Part of the axis of an embryo or stem of a seedling between the cotyledons and the radicle; usually identifiable as the region between the root collar and the base of the cotyledons.

hypogeal Seed germination in which the cotyledons remain below the ground. Compare *epigeal*.

in situ Literally, in place; to describe experiments conducted in the field or in their natural environment (*in vivo*), as opposed to in the laboratory (*in vitro*).

independent variable See *variable*.

insect-species complex A group of different insect species that feed on a single tree species.

integument The outer cell layer or layers that surround the ovule and give rise to the seed coat.

irradiance The electromagnetic radiant energy received per unit area of a plane surface. Compare *emittance*.

isopleth A line joining points of equal value; for example, a contour map consists of isopleths of elevation.

level of significance A statistical term expressing the probability that an apparently significant difference is not real but simply due to chance; the level is pre-set for an experiment, typically at 1% or 5%.

LFH layers Litter, fermentation, and humus layers of the soil profile, consisting of the surface organic layers (forest floor or duff in forest soils).

life table A tabulation of mortality and survivorship of a population; static, time-specific, or vertical life tables are based on a cross-section of a population at a given time; dynamic, cohort, or horizontal life tables are based on a cohort of organisms followed throughout life.

linear regression See *regression*.

longwave radiation Electromagnetic radiation with wavelength 3.0–100 μm; also known as thermal radiation.

male flowers (1) The male strobili of conifers that produce pollen. (2) The flowers of angiosperms that contain no female structures, only male (anthers).

MANOVA See multivariate analysis of variance.

mast year A year of unusually good seed production; generally applied to hardwoods.

maturation Final stage of seed development characterized by dehydration of seed tissues and, usually, the induction of dormancy.

mechanical site preparation The use of machines to prepare a site for reforestation; may consist of dragging anchor chains or shark-fin barrels, disc trenching, plowing, or mounding. See *site preparation*.

megagametophyte The nutritive tissue (1N) of gymnosperm seeds, which surrounds and nourishes the embryo. Often incorrectly called *endosperm*.

meristem Undifferentiated tissue that is capable of undergoing cell division; located in root and shoot tips where growth in length occurs in axillary buds of male and female cones, or in the secondary meristem tissue (cambium) where growth in girth occurs.

metafactor A complex factor or a set of independent variables which are tightly associated, and hence often treated as a single factor. See *factor*.

microclimate The small-scale climates of hill and hollow, field and forest; the physical environment of plant communities, insects, fish, and wildlife; may differ significantly from the general climate of the region. See *microsite*.

micropyle A minute opening into an ovule of an angiosperm plant through which the pollen grain normally passes to reach the egg cell; usually closed in the mature seed to form a superficial scar. See *ovule*.

microsite The specific spot or local habitat occupied by an organism; the environmental conditions sensed by an individual organism.

microsporophyll. The spore-producing structure of plants; in angiosperms, the stamen. See *male flowers*.

moisture content (mc) A measure of the amount of water present in a seed; can be expressed as a percentage of either fresh or dry weight.

monoecious Literally, *one house*. Describes plants in which both male (staminate) and female (pistillate) flowers are borne on the same plant. Compare *dioecious.*

multistage sampling Experimental design where samples are taken at successive layers of randomization. For example, two-stage sampling involves selection of a sample of secondary units from the primary units; three-stage sampling involves selection of a third level of samples from the secondary units; higher-order multistage designs are also possible.

multivariate analysis of variance (MANOVA) An extension of ANOVA with comparisons made on a group of dependent variables.

natural regeneration The renewal of a forested area by natural as opposed to human means (e.g., by seeds derived from adjacent stands, or by seeds transported by wind, birds, or animals).

nonlinear regression See *regression*.

nonparametric Statistical methods for analyzing data when a "classical" or specified distribution is inappropriate. See *normal distribution*. Compare *parametric*.

normal distribution A symmetrical, bell-shaped distribution curve, with the mean, median, and mode coinciding (see *central tendency* for definitions). Such data fulfills the requirements for analysis using *parametric* statistics.

normal probability plot A diagnostic plot used to check whether data (or residuals) are normally distributed. The plot is a graph of the cumulative distribution of the data (or residuals) on normal probability paper (paper scaled in such a way that the cumulative normal distribution plots as a straight line). See *normal distribution*.

nut Dry, indehiscent, one-seed fruit with a hard wall (e.g., fruit of *Quercus*).

nutlet A small nut or nut-like fruit (e.g., fruit of *Betula*).

orthodox A term to describe seeds that can be stored for long periods at low moisture content (5–10%) and below zero temperatures; this group includes all British Columbia conifer seeds, and many hardwoods. Compare *recalcitrant.*

ovule A female organ surrounded by integument, within which an egg cell (1N) is produced, and which, following fertilization, matures into a seed (2N).

parametric Statistical methods for analyzing data from a specified distribution. Compare *nonparametric*.

partial cutting Logging practices in which only certain individuals are removed from a stand of harvestable trees. Compare *clearcutting*.

Pearson product-moment correlation coefficient A statistic that characterizes the strength of the linear relationship between two variables; its square is equivalent to r^2 for simple linear regression.

percentile (e.g., *p*-th percentile) A value such that when data are ordered from smallest to largest, at least *p*% of the observations are at or below this value.

periodicity A cycle of time over which a phenomenon repeats itself. For example, many conifers do not produce collectable crops every year, but depending on the species, may only produce cones at 3–10 year intervals.

phenology Study of the relationship between seasonal climatic changes and periodic biological phenomena such as flowering, fruiting, leafing, growth flushing, and dormancy.

phenotype All characteristics—morphological, anatomical, and physiological—of an organism, determined by the interaction between the genotype and the environment. Compare *genotype*.

photoinhibition A reversible loss of photosynthetic capacity that occurs when a plant is exposed to excessive sunlight. Compare *sun scald*.

photosynthetically active radiation (PAR) Electromagnetic radiation in the wavelength band 400–700 nm, which contains the wavelengths absorbed by plants for photosynthesis.

phytochrome Protein-based plant pigment that exists in two interconvertible forms; it changes from one form to the other by absorption of red (660 nm) or far-red (730 nm) light.

pollination Process by which pollen in transferred from the male structure where it is produced to the female structure. In gymnosperms, pollen is dispersed by wind from male to female cones. In angiosperms, pollen may be wind dispersed, or carried by animals from the male to female flowers.

pome Many-seeded fruit of the apple family consisting of an enlarged fleshy receptacle surrounding the

pericarp; in *Malus* the pericarp is papery and fleshy; in *Crataegus* it is hard and stony.

post-dispersal To describe events occurring after dispersal of seeds from the mother tree. Compare *pre-dispersal*.

power A statistical term, expressing the probability of detecting a difference when in fact there is a difference.

precision The degree to which a set of estimates is closely grouped together. Compare *accuracy*.

pre-dispersal To describe events occurring while the seeds are still attached to the mother tree. Compare *post-dispersal*.

provenance (of seeds) The geographical area (latitude, longitude, and elevation) and environment to which the parent trees are native, and within which their genetic constitution has evolved through natural selection; their genetic origin. Compare *seed source*.

pseudoreplication When subsamples are statistically treated as experimental units, when, in fact, they are not. Compare *replication*. See *experimental unit*.

quantum An indivisible unit or discrete packet of energy.

quartiles The three values of a variable dividing a set of ordered data into quarters: the 25th, 50th, and 75th percentiles. See *percentile*.

r^2 and adjusted r^2 See *coefficient of determination*.

R50 See *germination rate*.

R50' See *germination speed*.

radiation or **radiant energy** Energy transferred through space in the form of electromagnetic waves or photons.

radicle Portion of the axis of an embryo from which the root develops.

radioactive Capable of giving off high-energy particles or waves, such as the alpha, beta, and gamma rays produced by disintegration of atomic nuclei; can be used as a tag to identify an object, such as a seed, for later recovery.

radioisotope (also radioactive isotope). An unstable isotope which, upon decay, can be detected with a *scintillometer*.

random factor See *factor*.

randomization In experimental design, the assignment of treatments to the experimental material in an unbiased manner.

recalcitrant A term to describe seeds that will not germinate unless they are stored at relatively high moisture content (>15%). Seeds of this type cannot be stored successfully for long periods (generally only several weeks to several months). Compare *orthodox*.

recruitment The successful transition or graduation of an organism from one age class or stage to another (as from seed to seedling, or from seedling to sapling), or the organisms that have made this transition.

reforestation Actions taken to re-establish continuous tree cover after mature trees have been harvested or otherwise lost.

regression A statistical technique for modelling the relationship between two or more variables; linear regression assesses the relationship between variables that can be depicted by a straight line; nonlinear regression assesses the relationship between variables without assuming a linear relationship between them. See *stepwise regression*.

remay Lightweight, nonwoven white fabric, often used as a horticultural row cover. The material allows air, water, and 80–90% of light to pass through.

repeated measures analysis A special ANOVA technique applicable to data that consist of measurements collected on the same experimental unit(s) at more

than one time. That is, experimental units measured at different times cannot be treated as replicates because they are not independent—in fact, they are likely autocorrelated. See *autocorrelation*.

replication In experimental design, the independent application of treatment levels to an experimental unit. Compare *pseudoreplication*.

residuals In statistical analysis, the difference between the observed and predicted values after a model has been fitted.

root elutriator Tool designed to separate roots from soil and which can be adapted to separate seeds from soil.

samara A dry, indehiscent, winged fruit, one-seeded as in *Fraxinus* and most conifers, or two-seeded as in *Acer*.

sample germination A method for determining the number and species of seeds found in a soil seed bank by counting germinants that emerge from soil samples placed in a controlled environment. Compare *direct count*.

Satterthwaite's approximation A method of constructing approximate F-tests.

scarification (1) of seeds, the process of abrading a seed coat to make it more permeable to water, either by mechanical means or by brief exposure to hot water or to strong acids such as sulphuric acid; (2) of seedbeds, a method of seedbed preparation in which patches of mineral soil are exposed through mechanical action.

scintillometer An instrument for measuring gamma radiation emitted by a radioactive substance. Can be used to locate seeds previously tagged with a radioisotope.

screefing Removing weeds and small plants together with most of their roots from the area immediately surrounding the planting hole.

secondary dispersal Movement of seeds (by wind, water, or animals) after they have already fallen from the parent plant.

seed bank See *canopy bank; soil seed bank*.

seedbed In natural regeneration, the surface or substrate on which seeds falls; in nursery practice, a prepared area over which seeds are sown.

seed coat The protective outer layer of a seed derived from the integument of the ovule.

seedlot A quantity of seeds of the same species, provenance, date of collection, and handling history, which is identified by a single number.

seed orchard A plantation of specially selected trees that is managed for seed production, usually for the purpose of genetic improvement.

seed production stand A forest stand reserved and managed as a source of seeds.

seed rain The overall input of seeds on a surface per unit area per unit time.

seed shadow The area of ground with a high density of dispersed seeds, centred on (or downwind from) a seed-producing individual or stand. See *dispersal*.

seed source The place (latitude, longitude, and elevation) from which seeds are collected; their physical source. The source of a seed collection may not be identical to its *provenance*.

seed trap A device designed to collect all the seeds landing in a defined area.

seed tree (1) A seed-bearing tree. (2) An even-aged silvicultural system in which a forest stand is regenerated by removing all trees from an area except for a small number of seed-bearing trees left singly or in small groups. See *silvicultural system; partial cutting*.

selection system A method of harvesting and regenerating a forest stand, which maintains an uneven-aged structure by removing some trees in all size classes, either singly or in small groups. See *silvicultural system; partial cutting*.

serotiny (adj. **serotinous**) A term to describe cones that remain closed on the tree (often for several years after maturity); some require heat (fire) to disperse their seeds.

shelterwood An even-aged silvicultural system designed to establish a new crop under the protection (overhead or side) of the old. See *silvicultural system*, *partial cutting*.

significance level See *level of significance*.

silvicultural system A process whereby forests are tended, (thinning, pruning, etc.) harvested, and replaced to produce a crop of timber and other forest products. The particular system is typically named by the cutting method used for regeneration.

single-tree selection harvesting See *selection system*.

site index The measure of the relative productive capacity of a site for a particular crop or stand, generally based on tree height at a given age.

site preparation A treatment, either mechanical, fire, chemical, or manual, to modify a site to provide favourable conditions for natural or artificial regeneration of the desired tree species.

site series See *biogeoclimatic ecosystem classification*.

slope The angle of the ground relative to horizontal, expressed in degrees or as a percentage of the run to the rise.

soil seed bank All viable seeds present on or under the surface of the soil.

solar noon The time of day at which the sun is at its highest point. For a specific location and date the information is available from website http://www.crhnwscr.noaa.gov/grr/sunlat.htm

solar radiation Electromagnetic energy from the sun in the 0–3000 nm wavelengths, of which the 300–3000 nm range reaches the earth's surface. It has direct and diffuse components. The former is radiation directly from the solar disc, and the latter radiation that has been scattered by the atmosphere. Syn. global radiation, shortwave radiation.

spatial statistics A branch of statistics for studying the spatial variation of positional (x-y coordinate) data. Many of the methods stem from or overlap with geostatistics.

Spearman's rank order correlation A correlation technique that measures the relationship between two variables, based on the rank order of the data. It assesses whether two variables have a strictly increasing or strictly decreasing relationship.

standard deviation A statistic that assesses the spread or variability of data about the mean; the square root of variance.

stepwise regression A statistical procedure for systematically reducing the number of independent variables required to model the dependent variable.

stocking A measure of the area occupied by trees, usually measured in terms of well-spaced trees per hectare, or basal area per hectare, relative to an optimum or desired level.

stratification A dormancy-breaking treatment in which seeds are exposed to moist, cold (2–5°C) conditions for several weeks (or months, depending on the species). Compare *warm stratification*.

stressing Attempts to induce tree seedling dormancy or enhanced seed production through the application of some kind of physiological stress (e.g., drought) or mechanical stress (e.g., partial girdling).

strobile (pl. **strobiles**) Spiky pistillate inflorescence of angiosperms or the resulting fruit. Syn. female *catkin*.

strobilus (pl. **strobili**) The male and female reproductive structures of gymnosperms. Syn. *cone*.

Student's t-test See *t-test.*

subsample Units within an experimental unit to be sampled for measurements. For example, if the experimental unit is a tree, the seeds from that tree could be a subsample. See *experimental unit.*

sunfleck A relatively small area of forest floor that receives direct-beam solar radiation through the interstices of overstorey foliage and branches in an otherwise closed forest canopy.

sun scald Damage to foliage and destruction of chlorophyll incurred through exposure to high light intensities when the plant is not acclimated to such conditions, also called photodamage. Compare *photoinhibition*.

t-distribution Distribution of the Student's *t*-test statistic; similar in shape to a *normal distribution*; useful for small samples.

t-test or **Student's t-test** A statistical test that assesses the differences between two groups by comparing the means.

terminal velocity The maximum velocity of a falling object, determined by the force of gravity and the shape of the object.

top height trees The largest dominant or codominant trees of the same species that are healthy, undamaged, and unsuppressed.

transformation A mathematical procedure for converting data to a different scale using one or more mathematical functions (e.g., square root, sine, natural log, exponential); often used to make the data more appropriate for a statistical test.

transpiration Release of water vapour from the aerial parts of a plant, primarily through the stomata.

Type I error In statistical analysis, the error of rejecting the null hypothesis (the hypothesis that the treatment has no effect) when it is in fact true; the probability is usually preset as the *level of significance* of the test.

Type II error In statistical analysis, the error of not rejecting the null hypothesis (the hypothesis that the treatment has no effect) when in fact it is false; the probability of avoiding which is the *power* of the test.

ultraviolet (UV) radiation Radiation in the waveband 200–400 nm. Since UV-C (200–290 nm) is filtered out by the atmosphere, UV radiation received at the earth's surface is in the 290–400 nm range.

uneven-aged Describes a forest, stand, or forest type in which relatively large age differences (>15–20 years) exist between individual trees within a stand; these age differences usually denote multiple *cohorts*. Compare *even-aged*.

variable An expression that can be assigned any of a set of values. A variable can be independent (causal) with levels set by the experimenter, or dependent (response) and responding to changes in the independent variable. The terms discrete, continuous, and categorical are used to describe data or a variable: discrete—having a distinct value, sometimes expressed in whole numbers (e.g., number of filled seeds); as opposed to continuous—able to take a continuum of values (e.g., percent germination, seed weight). Categorical describes information that has been grouped (e.g., age class, colour, species).

variance A statistic that measures the spread in the data; the square of standard deviation.

viable Alive; with respect to seeds, capable of germination and subsequent growth and development of the seedling.

vigour The combination of properties which enables seeds to germinate quickly under a wide range of environmental conditions, and which endows germinants with the ability to establish quickly and resist disease. Seeds that perform well under a wide range of environmental conditions are termed high vigour seeds and those that perform poorly are called low vigour seeds.

warm stratification A dormancy-breaking treatment of seeds in which moist seeds are held at warm temperatures (usually 20–25°C) for several weeks. The warm incubation period is usually followed by an incubation period at 2–5°C. See *stratification*.

wing loading A measure of the weight-to-area ratio of an airborne or falling object; equal to its mass times gravitational acceleration divided by its planform (projected, one-sided area).

z-test (z-score) The *z*-test and the *z*-score are examples of Wald statistics. They are computed by taking the estimated parameter, subtracting from it the parameter value under the null hypothesis, and then dividing this entire quantity by the approximated standard error of the estimated parameter. Under certain conditions, this statistic is asymptotically (i.e., large sample sizes) distributed with a standard normal distribution.

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The researches of many commentators have already thrown much darkness on this subject, and it is probable that, if they continue, we shall soon know nothing at all about it.
(Mark Twain)

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